From the Department of Biosciences and Nutrition
Karolinska Institutet, Stockholm, Sweden

SKIN CELL HETEROGENEITY AND DYNAMICS DURING MORPHOGENESIS, TISSUE HOMEOSTASIS, AND REGENERATION

Tina Jacob

Karolinska Institutet

Stockholm 2021

Cover illustration: The globe symbolizes the molecular mapping of skin tissue landscape. Each circle on the globe illustrates a single cell, with similar colors denoting similar transcriptomes. The Arctic Circle represents the interfollicular epidermis, which connects the small continent (a hair follicle-to-be), the medium-sized continent (a hair follicle in rest), and the large continent (a hair follicle actively producing hair). The ocean represents stromal cells that are crucial for tissue integrity and function. Artwork by Tina Jacob

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-153-4

Skin cell heterogeneity and dynamics during morphogenesis, tissue homeostasis, and regeneration

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Tina Jacob

The thesis will be defended in public at Karolinska Institutet,
Eva & Georg Klein Lecture Hall, Biomedicum,
Solnavägen 9, Campus Solna, Stockholm

Friday, April 23rd, 2021 at 9:30 AM

Principal Supervisor:
Dr. Maria Kasper
Karolinska Institutet
Department of Cell and Molecular Biology

Co-supervisor(s):
Prof. Rune Toftgård
Karolinska Institutet
Department of Biosciences and Nutrition

Dr. Marco Gerling
Karolinska Institutet
Department of Biosciences and Nutrition

Opponent:
Dr. Carlos Clavel
Agency for Science, Technology and Research (A*STAR)
Skin Research Institute of Singapore

Examination Board:
Dr. Qiaolin Deng
Karolinska Institutet
Department of Physiology and Pharmacology

Prof. Christos Samakovlis
Stockholm University
Department of Molecular Biosciences

Prof. Jonas Frisén
Karolinska Institutet
Department of Cell and Molecular Biology

"Poets say science takes away from the beauty of the stars - mere globs of gas atoms. I too can see the stars on a desert night and feel them. But do I see less or more? The vastness of the heavens stretches my imagination – stuck on this carousel my little eye can catch one - million - year - old light. A vast pattern – of which I am a part… What is the pattern, or the meaning, or the why? It does not do harm to the mystery to know a little about it."

Richard Feynman

ABSTRACT

Skin is our protective barrier against various environmental harms. For the skin to fulfill its crucial function, it relies on multiple cell types working in concert; but most importantly it relies on skin-resident epithelial stem cells. These cells ensure an intact barrier through constant replacement of the epidermis and they ensure proper hair production through cyclical regeneration of hair follicles. This combination of constant and cyclical renewal within one tissue makes skin a prime model system for the study of adult tissue stem cells.

The overall aim of this thesis was to transcriptionally dissect this well-established model system in a systematic and unbiased way. The majority of data presented in this thesis is based on the combination of single-cell RNA sequencing and *in situ* stainings of mRNA. This combination allows us to appreciate the genome-wide transcriptional heterogeneity while still being able to place the identified cell populations in their spatial tissue context.

In Paper I, the first whole-transcriptome study of skin at the single-cell level, we examined the vectors describing cellular heterogeneity within the epidermal compartment of mouse skin during its resting stage (telogen).

In Paper II, we expanded on this analysis by including full-thickness skin during rest (telogen) and growth (anagen). This allowed for an unbiased census of all major cell types contained in the skin, and it furthermore enabled us to study how skin achieves and accommodates hair growth.

In Paper III, we studied the role of dermal fibroblasts in early embryonic skin development. We uncovered unexpected heterogeneity among embryonic fibroblasts and explored their supportive functions for skin maturation. Moreover, we identified novel keratinocyte subpopulations and closely analyzed epidermal fate decisions.

In Paper IV, we monitored transcriptional adaptations of two distinct epidermal stem cell populations during their contribution to wound healing. This allowed us to answer fundamental questions about stem cell plasticity and the dynamics of cell adaptations following injury.

In sum, this thesis uncovers the dynamic and heterogeneous nature of mouse skin during adult tissue homeostasis, embryonic development, and tissue regeneration after injury. Most importantly, we provide new insights into how stem cell identity is shaped and how developmental as well as regenerative processes are orchestrated.

SCIENTIFIC PAPERS INCLUDED IN THIS THESIS

I. Simon Joost, Amit Zeisel, **Tina Jacob**, Xiaoyan Sun, Gioele La Manno, Peter Lönnerberg, Sten Linnarsson, and Maria Kasper. *Single-cell transcriptomics reveals that differentiation and spatial signatures shape epidermal and hair follicle heterogeneity*. Cell Systems 3, 3 (2016): 221-237.

II. Simon Joost*, Karl Annusver*, **Tina Jacob**, Xiaoyan Sun, Tim Dalessandri, Unnikrishnan Sivan, Inês Sequeira, Rickard Sandberg, and Maria Kasper. *The molecular anatomy of mouse skin during hair growth and rest*. Cell Stem Cell 26, 3 (2020): 441-457.

III. **Tina Jacob**, Karl Annusver, Paulo Czarnewski, Tim Dalessandri, Maria Eleni Kastriti, Chiara Levra Levron, Beate Lichtenberger, Giacomo Donati, Åsa Björklund, and Maria Kasper. *Fibroblasts govern the molecular design of early skin development*. Manuscript.

IV. Simon Joost, **Tina Jacob**, Xiaoyan Sun, Karl Annusver, Gioele La Manno, Inderpreet Sur, and Maria Kasper. *Single-cell transcriptomics of traced epidermal and hair follicle stem cells reveals rapid adaptations during wound healing*. Cell Reports 25, 3 (2018): 585-597.

* These authors have contributed equally.

ADDITIONAL SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

I. Anja Füllgrabe, Simon Joost, Alexandra Are, **Tina Jacob**, Unnikrishnan Sivan, Andrea Haegebarth, Sten Linnarsson, Benjamin D. Simons, Hans Clevers, Rune Toftgård, and Maria Kasper. *Dynamics of Lgr6^{+} progenitor cells in the hair follicle, sebaceous gland, and interfollicular epidermis*. Stem Cell Reports 5, 5 (2015): 843-855.

II. Ka-Wei Mok, Nivedita Saxena, Nicholas Heitman, Laura Grisanti, Devika Srivastava, Mauro J. Muraro, **Tina Jacob**, Rachel Sennett, Zichen Wang, Yutao Su, Lu M. Yang, Avi Ma'ayan, David M. Ornitz, Maria Kasper, and Michael Rendl. *Dermal condensate niche fate specification occurs prior to formation and is placode progenitor dependent*. Developmental Cell 48, 1 (2019): 32-48.

III. Xioayan Sun*, Alexandra Are*, Karl Annusver°, Unnikrishnan Sivan°, **Tina Jacob**, Tim Dalessandri, Simon Joost, Anja Füllgrabe, Marco Gerling, and Maria Kasper. *Coordinated hedgehog signaling induces new hair follicles in adult skin*. Elife (2020): 9:e46756.

IV. Anton J.M. Larsson, Björn Reinius, Christoph Ziegenhain, **Tina Jacob**, Tim Dalessandri, Gert-Jan Hendriks, Maria Kasper, and Rickard Sandberg. *Transcriptional bursts explain autosomal random monoallelic expression and affect allelic imbalance*. PLoS Computational Biology 17, 3 (2021): e1008772

* These authors have contributed equally.
° These authors have contributed equally.

CONTENTS

LIST OF ABBREVIATIONS

AGM	Aorta, gonads, mesonephrons
BBKNN	Batch balanced k-nearest neighbors
bDNA	Branched DNA
BMP	Bone morphogenetic protein (cell signaling)
CD	Cluster of differentiation (cell signaling)
cDNA	Complementary DNA
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DC	Dendritic cell
DETC	Dendritic epidermal T cell
DNA	Deoxyribonucleic acid
DWAT	Dermal white adipose tissue
E (e.g., E14.5)	Embryonic day
ECM	Extracellular matrix
EDA	Ectodysplasin (cell signaling)
EGF	Epidermal growth factor (cell signaling)
EGFP	Enhanced green fluorescent protein
EMP	Erythro-myeloid progenitors
EPU	Epidermal proliferation unit
FACS	Fluorescence-activated cell sorting
FAK	Focal adhesion kinase (cell signaling)
FGF	Fibroblast growth factor (cell signaling)
FISH	Fluorescent *in situ* hybridization
HCR	Hybridization chain reaction
HGF	Hepatocyte growth factor (cell signaling)
HSC	Hematopoietic stem cell
HVG	Highly variable gene
IFE	Interfollicular epidermis
IL	Interleukin (cell signaling)
IRES	Internal ribosome entry site
IRS	Inner root sheath

ISH	*In situ* hybridization
ISS	*In situ* sequencing
KGF	Keratinocyte growth factor (cell signaling)
kNN	k-nearest neighbors
LTMR	Low-threshold mechanoreceptors
MDP	Macrophage and dendritic cell progenitors
MHC	Major histocompatibility complex (cell signaling)
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B cells (cell signaling)
ORS	Outer root sheath
P (e.g., P5)	Postnatal day
PCA	Principal component analysis
PCM	Panniculus carnosus muscle
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor (cell signaling)
PPAR	Peroxisome proliferator-activated receptor (cell signaling)
PSU	Pilosebaceous unit
RCA	Rolling circle amplification
RNA	Ribonucleic acid
RT	Reverse transcription
scRNA-seq	Single-cell RNA sequencing
SG	Sebaceous gland
SHH	Sonic Hedgehog (cell signaling)
smFISH	Single-molecule fluorescent *in situ* hybridization
SNP	Single nucleotide polymorphism
SWAT	Subcutaneous white adipose tissue
t-SNE	t-distributed stochastic neighbor embedding
TAZ	WW domain-containing transcription factor (cell signaling)
TGF	Transforming growth factor (cell signaling)
TMX	Tamoxifen
UMAP	Uniform manifold approximation and projection

UMI	Unique molecular identifier
VEGF	Vascular endothelial growth factor (cell signaling)
WIHN	Wound-induced hair neogenesis
WNT	Wingless and Int-1 (cell signaling)
YAP	Yes-associated protein (cell signaling)

1 INTRODUCTION

1.1 SKIN AS A MODEL SYSTEM FOR ADULT TISSUE STEM CELLS

The skin is the largest organ of the mammalian body. As the outermost barrier, it offers protection against the external environment including ultraviolet radiation, physical trauma, and pathogens. At the same time, it limits transcutaneous water loss and ensures thermoregulation and insulation. To ensure tissue integrity and functionality, skin cells have to be constantly replaced by adult tissue stem cells [reviewed in (Fuchs, 2007; Hsu et al., 2014a)].

The existence of adult tissue stem cells was first proven by Till, McCulloch and others in the 1960s when they identified the hematopoietic stem cell that maintains the entire blood-lineage throughout a lifetime (Becker et al., 1963; Till and McCulloch, 2012). In the meantime, adult tissue stem cells have been identified in many organs including the brain (Altman and Das, 1965; Temple, 1989), intestine (Barker et al., 2007; Bjerknes and Cheng, 1999), mammary gland (Kordon and Smith, 1998; Smith and Medina, 1988), and skin (Potten and Morris, 1988) including hair follicles (Cotsarelis et al., 1990; Rochat et al., 1994; Tumbar et al., 2004).

While the traditional, hematopoietic stem cell-derived adult tissue stem cell paradigm is based on rare, quiescent stem cells, many years of research have uncovered that tissue stem cells constitute heterogenous populations that can include rare or abundant, quiescent or cycling, symmetrically or asymmetrically dividing cells which moreover display varying self-renewing capacities. However, they are all unified by their functional ability to maintain tissue homeostasis and to restore tissue integrity after injury [reviewed in (Clevers, 2015)].

For stem cells to meet the needs of a tissue, the processes of self-renewal and differentiation have to be tightly controlled. This regulation relies on extracellular cues which are discussed further in *1.3.4 The concept of stem cells and their niches*, as well as intracellular mechanisms including the portioning of cytoplasmic determinants among daughter cells, transcriptional regulation through a network of transcription factors, post-transcriptional regulation, and epigenetic regulation at the level of chromatin [reviewed in (Barresi and Gilbert, 2020)].

The skin with its hair follicles and interfollicular epidermis is particularly suited to the study of adult tissue stem cells – not just because of their unique accessibility for investigation. The interfollicular epidermis represents a stereotypic stratified tissue with the constant replacement of post-mitotic suprabasal cells by proliferating basal cells. In contrast, the hair follicles undergo cyclical renewal including sequential phases of growth (termed ‘anagen’), regression (termed ‘catagen’) and rest (termed ‘telogen’), that makes it possible to study the regulation of stem cell quiescence and activation in the context of a complete mini-organ. Furthermore, the hair follicle displays a well-defined, compartmentalized anatomy with epithelial and mesenchymal compartments as well as distinct stem cell populations that differ in their location, morphology, molecular markers, and function [reviewed in (Mesa et al., 2015a)]. Finally, it has been proven that the mechanisms and signaling pathways identified in the skin

are conserved among many other tissues [reviewed in (Cunha and Hom, 1996; Ribatti and Santoiemma, 2014)].

This thesis describes the systematic analysis of this well-established model system for adult tissue stem cells using single-cell transcriptomics. Notably, the papers included in this thesis are based on mouse skin, which is also the focus of the introductory chapter. While mouse skin is a valid model for human skin, there are important differences that will be discussed in section *1.9 Comparison of human and mouse skin*.

1.2 SKIN ANATOMY

Skin can be subdivided into three main compartments: the epidermis and the underlying, supportive dermis and hypodermis (Figure 1A). The epidermis and dermis are separated by the basement membrane, a sheet of fibers that is rich in collagens (particularly type 4 and 17), and laminins [reviewed in (Hsu et al., 2014a)].

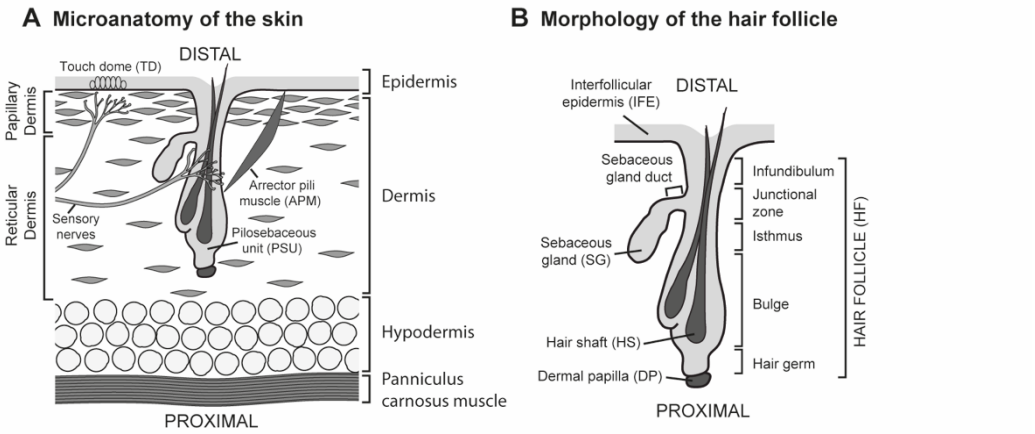

Figure 1: Anatomy of mouse skin (A) and the telogen hair follicle (B) [adapted from (Joost, 2019)]

The epidermis consists of specialized epithelial cells, so-called keratinocytes, that build the multi-layered outer skin barrier, as well as the hair follicles which produce hair, and the sebaceous gland. Telogen hair follicles display a particular architecture containing the following compartments from distal to proximal (Figure 1B): (a) the infundibulum, which connects the interfollicular epidermis (i.e., the epithelial tissue in-between hair follicles; IFE) to the hair follicle, (b) the junctional zone, where the sebaceous gland opens into the hair canal, (c) the isthmus, the most narrow segment of the hair follicle, (d) the bulge, a two-layered structure containing post-mitotic cells as well as undifferentiated stem cells, and (e) the hair germ, being the connecting link to the stromal niche. Just below the hair germ there is the specialized dermal signaling center called dermal papilla, which is a condensation of mesenchymal cells and acts as a key component of the hair follicle-associated stromal niche. Notably, hair follicles in the skin are oriented in a uniform manner with dermal papillae always pointing in a rostral direction and hair shafts growing in a caudal direction [reviewed in (Hsu et al., 2014a)].

Four different types of hair follicles exist in dorsal mouse skin (guard, awl, auchene, and zigzag hair follicles). While their gross anatomy is the same, they differ at the molecular level and the architecture of the produced hair shaft (Schlake, 2007).

Besides the hair follicle, the pilosebaceous unit contains the sebaceous gland, which is a holocrine gland that produces sebum to lubricate and acidify the skin surface [reviewed in (Niemann and Horsley, 2012)]. The arrector pili muscle is a smooth muscle that attaches to the hair follicle bulge and can erect hair in a setting commonly known as 'goose bumps' (Müller-Röver et al., 2001).

Mouse skin also contains a number of other glands, such as eccrine sweat glands, lacrimal glands, and mammary glands [reviewed in (Dhouailly and Oftedal, 2016)].

In addition to the discussed epidermal components, skin heavily relies on the cellular and non-cellular components of the underlying dermis and hypodermis. A plethora of non-epidermal cell types reside in the skin and support its function. The heterogeneity among those cells as well as the nature of their support to the epidermal compartment will be discussed in detail in section *1.6 Non-epidermal skin compartments*.

1.3 THE EPIDERMAL COMPARTMENT

1.3.1 Maintenance of the interfollicular epidermis

While it is well understood that basal keratinocytes are critical players for maintenance of the interfollicular epidermis, there are two major opposing views on how exactly mouse interfollicular epidermis is maintained: the hierarchical model, which assumes that rare, quiescent stem cells are hidden between the abundance of proliferative progenitors in the basal interfollicular epidermis, and the neutral competition model, which doubts the existence of one or more privileged, distinct stem cell populations in the interfollicular epidermis and instead proposes an equipotent pool of progenitor cells.

The hierarchical model was originally proposed by Mackenzie and builds on a strict hierarchy of long-lived, slow-cycling stem cells that divide asymmetrically to generate a stem cell daughter and a transit-amplifying daughter, which eventually differentiates (Mackenzie, 1970). Potten built his theory of the 'epidermal proliferative unit' (EPU) on this hierarchical model. He posited that the epidermis is organized into hexagonal units, which are based on about ten basal cells with one central dormant stem cell per unit (Potten, 1974). Many years later Mascré et al. described that the basal layer of tail epidermis contains slow-cycling stem cells and fast-dividing progenitor cells (Mascré et al., 2012). This model was later revisited by Sada et al. They came to the conclusion that instead of a stem cell and a progenitor population, there are two mutually independent stem cell populations that proliferate at different rates. They occupy different regions under homeostatic conditions (scale and interscale region, respectively), but can renew each other's territory upon wounding or selective killing (Sada et al., 2016).

In contrast, the neutral competition model posits that basal epidermal progenitors all have the same potential, and that cell fate choice is a stochastic process. Cell divisions can result in two progenitor cells, a progenitor and a post-mitotic cell, or two post-mitotic cells that eventually differentiate and leave the basal layer of the epidermis. This model is linked to three major hallmarks: over time population asymmetry leads to an increase in the average clone size, a decrease in the number of clones, and a constant overall size of the progenitor population. The neutral competition model was first described by Clayton et al. in tail skin (Clayton et al., 2007), and was later proven to hold true for ear skin (Doupé et al., 2010), paw skin (Lim et al., 2013), dorsal skin (Füllgrabe et al., 2015), as well as other tissues such as the intestine (Snippert et al., 2010a) and esophagus (Doupé et al., 2012). Moreover, it seems like an equipotent keratinocyte population can even explain postnatal growth of tail and paw epidermis (Dekoninck et al., 2020).

Regardless of the model that best describes interfollicular epidermis maintenance, basal keratinocytes have a number of features that are crucial for them to maintain their basal, undifferentiated identity. Their attachment to the basement membrane via hemidesmosomes is of utmost importance. This was reinforced by recent findings that basal keratinocytes expressing high levels of the hemidesmosome component COL17A1 have a competitive advantage and loss of COL17A1 with aging leads to skin atrophy and fragility (Liu et al., 2019). Moreover, basal keratinocytes actively contribute to the formation of the basement membrane via the secretion of basement membrane components such as laminins (mainly LAMA5) and integrins (mainly α3β1 and α6β4) (Fujiwara et al., 2011). Transcriptomic and epigenomic studies have furthermore revealed a gene signature that is important for the maintenance of basal identity. This signature includes a number of transcription factors (*Zbed2*, *Etv4*), signaling molecules (*Wnt7b*, *Fgfr2*), and cell adhesion molecules (*Cdh3*, *Fat1*, *Dsg3*), among others (Finnegan et al., 2019; Lim et al., 2013).

1.3.2 Epidermal stratification

The multi-layered interfollicular epidermis consists of four histologically and molecularly distinct layers (Figure 2): the basal layer (stratum basale), the spinous layer (stratum spinosum), the granular layer (stratum granulosum), and the cornified layer (stratum corneum). This stratification is fueled by proliferating progenitor cells in the basal layer, whose daughter cells gradually differentiate towards the epidermal surface and eventually are shed in a process called ‘desquamation’. During homeostasis, proliferation is restricted to basal cells (*Krt5*$^+$/*Krt14*$^+$) that are anchored to the basement membrane. Upon initiated differentiation, keratinocytes enter the spinous layer (*Krt1*$^+$/*Krt10*$^+$) and later they progress to the granular layer (*Lor*$^+$/*Flg*$^+$). At the surface of the mammalian epidermis, there is the stratum corneum containing enucleated, terminally differentiated cells whose plasma membrane has been replaced by a cornified envelope consisting of cross-linked proteins and lipids [reviewed in (Fuchs, 1990)].

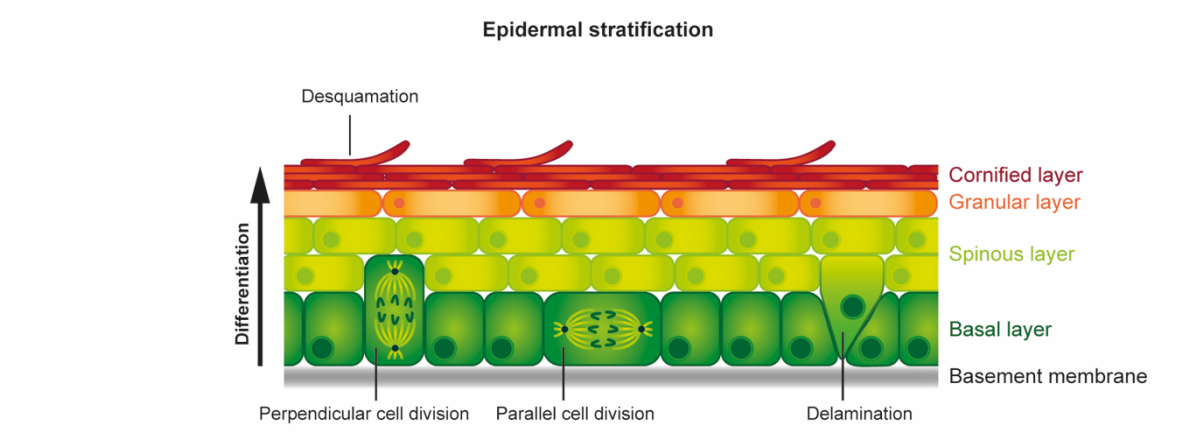

Figure 2: Layers of the stratified epidermis and modes of basal cell division during embryogenesis and homeostasis.

How exactly epidermal stratification is achieved is a matter of ongoing debate. The traditional view assumes that asymmetric divisions with spindle orientation perpendicular to the basement membrane place one daughter cell to the basal layer while the other one is placed directly to the suprabasal layer (Lechler and Fuchs, 2005). This model is based on the analysis of embryonic skin. More recently, it has been suggested that the majority of basal keratinocytes in the embryo rather divide in parallel to the basement membrane (Damen et al., 2020; Miroshnikova et al., 2018). Only upon basal layer crowding, individual basal cells would then initiate differentiation and delamination (Miroshnikova et al., 2018). Most likely, both mechanisms operate in parallel and they only shift their balance. Evidence suggests that the single-layered embryonic epidermis is dominated by parallel divisions, but that the proportion of perpendicular divisions increases significantly upon the initiation of stratification around embryonic day 14 (E14) when a large amount of suprabasal cells has to be generated from scratch. Once adult homeostasis is reached and only a few suprabasal cells have to be replaced upon shedding, the epidermis seems to return to mostly parallel divisions (Clayton et al., 2007; Ipponjima et al., 2016). Strikingly, balancing the two modes can even serve as a means of cell competition (Ellis et al., 2019; Liu et al., 2019).

It furthermore remains under investigation as to whether cell divisions precede or follow differentiation events. However, a recent study based on intravital-imaging convincingly shows that in the adult epidermis, the demand for differentiated cells drives stem cell divisions in the basal layer, i.e., differentiation and delamination precede cell division by one to two days. This mechanism suggests that the fates of neighboring cells are balanced. Once a cell has differentiated and delaminated, neighboring cells increase in size to fill up the empty spot and eventually divide (Mesa et al., 2018). It remains unclear though how the neighboring cell senses the empty spot in the first place and how the size increase results in cell division. One possible explanation was recently provided by Xie and colleagues who revealed how epidermal keratinocytes induce cell division once a certain cell volume is reached (Xie and Skotheim, 2020).

It has also been a long-standing question as to whether differentiation is the cause or consequence of delamination (Cockburn et al., 2021; Watt and Green, 1982). However, recent

evidence clearly supports that the transcriptional differentiation program is initiated prior to delamination. Using immunostaining, genetic mouse models, as well as transcriptomics, several studies on adult and postnatal mouse skin have shown the existence of interfollicular keratinocytes that display a basal-differentiated double signature. Cockburn and colleagues could even show that some of those cells still possess the ability to divide (Aragona et al., 2020; Braun et al., 2003; Cockburn et al., 2021; Doupé et al., 2010; Lin et al., 2020; Schweizer et al., 1984).

1.3.3 Stem cell heterogeneity and plasticity in the resting hair follicle

Mammalian epithelia are governed by two general principles: (1) within a single epithelial tissue heterogeneous stem cells can co-exist (stem cell heterogeneity), and (2) stem cell populations might exhibit a wider differentiation potential compared to homeostasis when faced with a tissue regeneration setting (stem cell plasticity) [reviewed in (Donati and Watt, 2015)].

As the mouse hair follicle is a widely used model system for adult tissue stem cells, many different stem cell populations have been identified. The first stem cells to be discovered in the hair follicle were slowly-cycling, label-retaining cells located in the outer bulge that fit the traditional hematopoietic stem cell-derived understanding of adult tissue stem cells (Cotsarelis et al., 1990; Rochat et al., 1994). They are marked for example by *Krt15* and *Cd34* expression and can regenerate all components of the hair follicle in reconstitution assays (Morris et al., 2004; Trempus et al., 2003). Other genes linked to the bulge identity include *Sox9*, *Lhx2*, *Nfatc1*, *Tbx1*, and *Tcf3* (Chen et al., 2012; Horsley et al., 2008; Nguyen et al., 2006; Nowak et al., 2008; Rhee et al., 2006; Vidal et al., 2005). Bulge stem cells are kept quiescent through paracrine signaling (FGF18, BMP6) by *Krt6*$^{+}$ post-mitotic cells in the inner bulge (Pasolli, 2011). However, in the past decade a whole 'zoo' of stem cell populations and their according marker genes have been identified (Figure 3) starting with *Lgr5* in 2008 (Jaks et al., 2008), *Lrig1* in 2009 (Jensen et al., 2009), *Lgr6* in 2010 (Snippert et al., 2010b), and *Gli1* in 2011 (Brownell et al., 2011), among others. Notably, these non-bulge stem cells are marked by significantly higher proliferation rates [reviewed in (Jaks et al., 2010; Schepeler et al., 2014)].

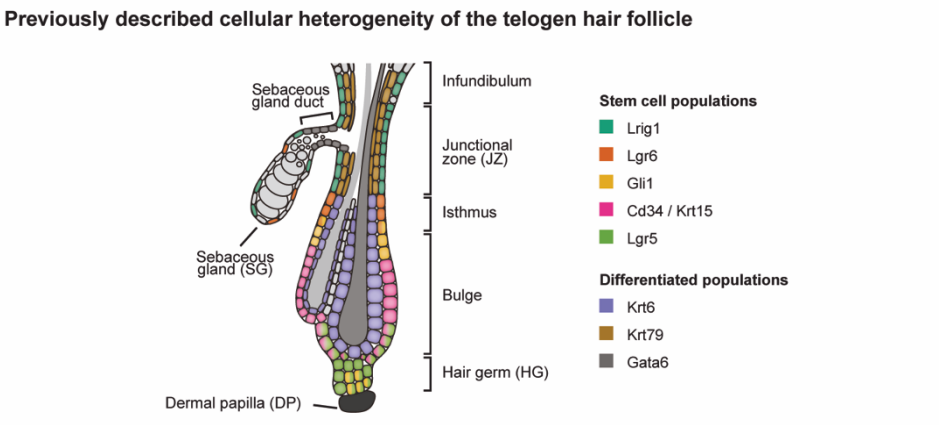

Figure 3: Previously identified stem cell populations as well as differentiated populations in the telogen hair follicle [adapted from (Joost, 2019)].

During homeostasis, the different stem cell populations display distinct localizations and contribution patterns (i.e., compartmentalization of skin). The exact mechanisms for establishing compartmentalization in the skin remain to be elucidated. There are, however, a number of factors that have been implicated in maintaining structural integrity in other organs or model systems. These factors include mechanical forces, cell sorting based on differential expression of adhesion or signaling molecules, and restricted movement by a zone of non-proliferating cells [reviewed in (Dahmann et al., 2011)].

Even though the compartments are very stable under homeostatic conditions, there are a number of settings that can allow the cell populations to become more plastic and overcome the restrictions that usually keep them within their compartment. In doing so, stem cells that normally occupy a certain niche, can move to and populate a different niche. A few examples of such plasticity are: (a) *Lgr5*$^{+}$ progeny that is usually restricted to the lower hair follicle but can migrate out of the hair follicle upon wounding to contribute to wound interfollicular epidermis (Kasper et al., 2011); (b) bulge cells that can be replaced by non-bulge cells after laser ablation of the whole bulge (Rompolas et al., 2013); and (c) *Lrig1*$^{+}$ progeny that is usually restricted to the upper hair follicle and sebaceous gland but can contribute to all epidermal compartments in a skin reconstitution assay (Jensen et al., 2009; Page et al., 2013).

Even though this multitude of epidermal populations with stem cell potential has been identified, some of the major questions that remain are: Besides their differential contribution pattern, what cellular, molecular and functional factors set those populations apart? On the other hand, what features unite them and specify stemness? Comparative approaches to date have been complicated by the fact that results for the different stem cell populations have been obtained from different studies with varying methods and varying study designs. Moreover, initial transcriptional analysis was limited by the fact that bulk RNA sequencing samples often contain a mixture of molecularly different cells and thus cannot be interpreted unequivocally.

1.3.4 The concept of stem cells and their niches

A stem cell's ability to regenerate tissues in homeostasis and under stress heavily relies on its ability to switch between quiescence and proliferation. A supportive microenvironment, also called niche, can help the stem cell to properly balance quiescence and proliferation by conveying the particular needs of a tissue via suitable cues. For epidermal stem cells, such extrinsic cues include homotypic cell interactions (e.g., interaction with the epithelial neighbors), heterotypic cell interactions (e.g., interactions between epithelial cells and fibroblasts or immune cells), interactions with the extracellular matrix (e.g., basal cells on the basement membrane), oxygen, membrane-bound factors, soluble factors, mechanical forces, and the complex tissue topography of the epidermis [reviewed in (Watt, 2016)]. Strikingly, it has been shown that even stem cell progeny can signal back to the stem cell and become part of its niche (Hsu et al., 2014b).

The importance of the individual niche components in the skin will be discussed in more detail in section *1.6 Non-epidermal skin compartments*.

1.4 HAIR CYCLING

While the interfollicular epidermis is constantly renewed, the hair follicle undergoes a cyclical regenerative program which is characterized by bouts of growth (anagen), regression (catagen), and rest (telogen) (Figure 4A). The hair cycle follows a stereotypic time course with distinct morphological and molecular characteristics that have been classified in detail by Müller-Röver et al. (Müller-Röver et al., 2001). As the initial two hair cycles in postnatal mouse skin are synchronized (Paus, 1998), hair cycling is amongst the best-studied regenerative processes in the mammalian body [reviewed in (Hsu et al., 2014a)].

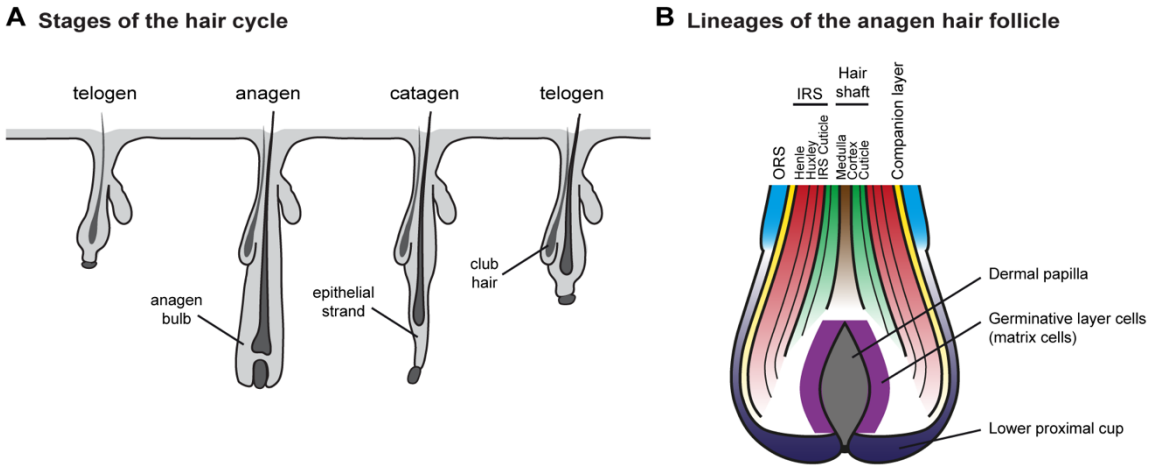

Figure 4: (A) Hair follicle morphology during the different hair cycle stages. (B) Microanatomy of the anagen hair bulb including the different lineages. ORS: Outer root sheath. IRS: Inner root sheath [adapted from (Joost, 2019)].

Telogen

Telogen marks the energy-saving default state of mouse skin, in which the hair follicle is at its minimal size and retains the previously produced hair shafts [reviewed in (Geyfman et al., 2015)]. Right after a completed growth phase, hair follicles enter so-called refractory telogen. They are then unable to respond to external anagen-inducing stimuli in order to prevent excessive regeneration. Hair follicle stem cells stay quiescent due to inhibitory signals emanating from subcutaneous fat (BMP2), dermal fibroblasts (BMP4), and the inner bulge (BMP6, FGF18) [(Hsu et al., 2011; Plikus et al., 2008), and reviewed in (Plikus and Chuong, 2014)]. After a while, hair follicles enter competent telogen which is marked by a shifted balance from inhibitory to activating signals and by maximal susceptibility towards anagen-inducing signals (Plikus et al., 2008).

Early anagen

Interactions between the hair follicle and the dermal papilla are both sufficient and necessary for the initiation of anagen. This was concluded from studies that on the one hand stalled hair growth by ablating dermal papillae and on the other hand successfully induced hair growth by transplanting dermal papillae (Jahoda et al., 1984; Rompolas et al., 2012). Dermal papilla-derived signals involved in anagen initiation include FGF7, FGF10, TGFβ2, and BMP

inhibitors (SOSTDC1, BAMBI, NOGGIN) (Greco et al., 2009; Hsu et al., 2014b; Oshimori and Fuchs, 2012). A tight control, both temporally and locally, of Bmp as well as Wnt signaling is not only important to ensure proper anagen initiation but is also crucial for proper anagen progression (Genander et al., 2014).

Hsu et al. characterized the detailed sequence of signaling events upon anagen entry (Anagen I-III). The resulting model is based on the notion that hair germ stem cells are most sensitive to activating signals from the dermal papilla. Once activated, hair germ cells initiate the production matrix progenitors. These, in turn, provide SHH to the underlying dermal papilla as well as to the overlying bulge stem cells, which promotes their self-renewal and sustains the growth of the bulge-derived outer root sheath (ORS). Because the matrix progenitor-pool follows the growing anagen hair follicle, pro-proliferative signals for the bulge eventually stop, which prevents cellular exhaustion and secures subsequent hair cycles. In the meanwhile, matrix progenitors continue to stay in contact with the dermal papilla which promotes their proliferation (Hsu et al., 2014b; Rompolas et al., 2013).

Mature anagen

Mature anagen (Anagen IV-VI) is characterized by a massively enlarged hair follicle with the so-called anagen bulb extending into the deep layers of skin. The anagen hair follicle is enveloped by the ORS and it progressively engulfs the dermal papilla [reviewed in (Alonso and Fuchs, 2006; Schneider et al., 2009)]. The anagen bulb contains matrix cells (also called germinative layer cells). Matrix cells are epidermal cells that are located at the lower end of the hair bulb directly juxtaposed to the dermal papilla and give rise to seven morphologically and molecularly distinct layers: the companion layer that separates the outer and inner root sheath (IRS), the three IRS layers (Henle, Huxley, and IRS cuticle) that form the hair canal that guides the growing hair, and the three layers of the actual hair shaft (hair shaft cuticle, cortex, and medulla) (Figure 4B) [reviewed in (Zhang and Hsu, 2017)]. It has been shown that matrix cells divide asymmetrically to renew their pool while also producing differentiating inner lineage cells (Legué and Nicolas, 2005; Yang et al., 2017).

There is an ongoing dispute about the dynamics of matrix cell differentiation and lineage specification. A number of studies have convincingly shown that the fate of matrix cells becomes constrained as anagen advances and that more distal cells preferentially give rise to hair shaft cells while more proximal cells rather produce companion layer and IRS (Legué and Nicolas, 2005; Legué et al., 2010; Mesler et al., 2017; Sequeira and Nicolas, 2012). Yang et al. proposed that matrix cells consist of seven uni-lineage progenitors that are spatially arranged along the dermal papilla. They report that these seven progenitors already display a molecular signature that reflects their differential priming for differentiation into one of the seven lineages (Yang et al., 2017). However, more recently Xin et al. showed that the fates of matrix cells residing at the different positions along the dermal papilla are not yet fixed. While matrix cells at specific positions along the dermal papilla give preferential rise to specific lineages, they can still undergo dynamic relocation. As they change their position along the dermal papilla, they also change their differentiation outcome. It is noteworthy though, that relocation only ever

happens in an upward direction (proximal to distal) (Xin et al., 2018). How these seemingly contradictory findings go together remains to be elucidated.

Catagen

Returning from the enlarged anagen hair follicle to the small telogen hair follicle requires that hair follicles undergo a regression phase called catagen. Among the inductive signals are dermal papilla-derived TGFβ and Wnt inhibitors such as DKK2 and NOTUM (Harshuk-Shabso et al., 2020), and indeed, ablated dermal papillae prevent hair follicle regression (Mesa et al., 2015b). During catagen, most cells are eliminated either via terminal differentiation of suprabasal cells or apoptosis of basal cells. Interestingly, epithelial cellular debris are not cleared by professional phagocytes but rather by epithelial neighbors (Mesa et al., 2015b). Some of the upper ORS cells are spared from the cleansing and form a new bulge and hair germ that can sustain the next hair cycle. This leaves the adjacent old bulge, also called club hair, as a stem cell reservoir and as an anchor for the hair produced in the previous cycle. It has furthermore been suggested that some of the lower ORS cells are kept to form the *Krt6*$^{+}$ inner layer of the new bulge (Hsu et al., 2011; Mesa et al., 2015b). Recently, the dermal sheath, which lines the anagen hair follicle and contains a smooth muscle contractile machinery, has been identified as the key driver of tissue regression and niche relocation. By contracting, the dermal sheath simultaneously pushes up the hair shaft and pulls up the epithelial strand (Heitman et al., 2020).

1.5 EPIDERMAL DEVELOPMENT

1.5.1 Specification of epidermis and initiation of epidermal stratification

Skin is a tissue that is specified early during mammalian embryonic development. Epidermis develops from the ectoderm. Ectoderm becomes committed to an epidermis fate upon Wnt pathway activation, which in turn stops ectodermal cells from responding to Fgf signaling. As a consequence, the ectoderm starts expressing BMPs which promote epidermal fate. Conversely, ectoderm exposed to SHH takes on a neural fate [reviewed in (De Falco et al., 2014)].

Once the epidermis is specified, epidermal stratification is executed within 9-10 days (approximately E9.5 to E18.5) (Figure 5). Initial induction of TAp63 expression in the surface ectoderm induces *Krt14* expression (E9.5) which is very specific to embryonic and mature basal keratinocytes. After this initial commitment, a second layer of squamous cells called periderm is established. This layer prevents pathological epithelial adhesions during embryogenesis and it is shed before birth when the epidermal barrier has matured (M'Boneko and Merker, 1988; Richardson et al., 2014). Next, DNp63 and Notch signaling promote the formation of an intermediate cell layer by induction of *Krt1* in suprabasal keratinocytes (E14.5). Subsequent maturation of intermediate cells into spinous cells is associated with reduced cell-cycle activity (E15.5). Continued differentiation partially depends on Ca^{2+} signaling and produces granular keratinocytes (E16.5). Finally, barrier formation requires

altered cell adhesions as well as the upregulation of genes involved in lipid synthesis and metabolism (E18.5) [reviewed in (Koster and Roop, 2007)].

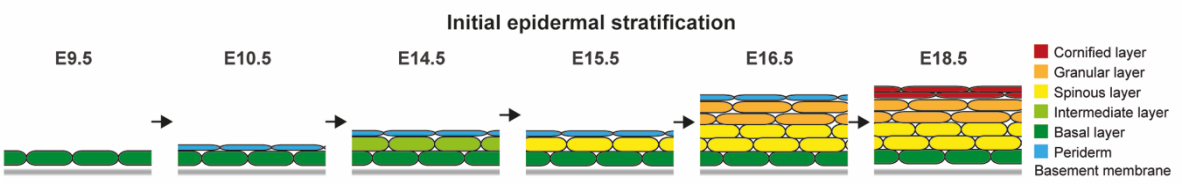

Figure 5: Embryonic, epidermal stratification from specification of the epidermis until acquisition of a terminally differentiated layer.

1.5.2 Hair follicle development

Hair follicle development in mouse, dorsal skin occurs in waves. At E14.5 the first wave of hair placodes appears, and those placodes develop into guard hairs which comprise 1-5 % of the adult mouse coat. The second wave starts at E16.5 and results in awl and auchene hairs that make up about 20 % of the adult coat. Finally, the vast majority of the adult coat is created at E18.5 when the third wave of hair placodes gives rise to zigzag hairs [reviewed in (Sennett and Rendl, 2012)].

Among these three waves, the first wave is the best-studied as there is no cross-contamination yet between the different waves which may confound interpretations (Figure 6). Therefore, the following section will focus on primary hair placodes (epithelial thickenings that give rise to guard hair follicles) and primary dermal condensates (mesenchymal condensations that give rise to the guard hair dermal papilla). Early primary placodes can be identified around E13.5 based on the expression of *Axin2*, *Krt17*, and *Fgf20*. Morphologically they become distinguishable at E14.0 concomitant with focal upregulation of placode markers including *Wnt10b* and *P-cadherin*. At E14.5 placodes have reached their full thickness, and the majority of hair follicle induction studies have been conducted at this time point [reviewed in (Ahtiainen et al., 2014)].

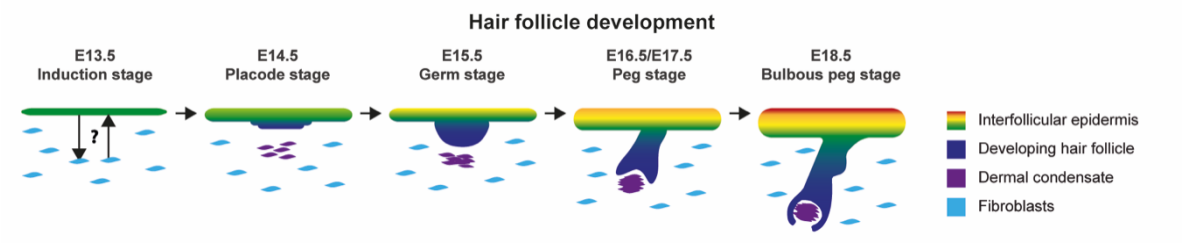

Figure 6: Embryonic hair follicle development from induction until bulbous peg state (embryonic age corresponds to 1st wave).

Hair follicle induction, similar to the induction of other ectodermal appendages (tooth, feather, cutaneous glands) and even sensory placodes (olfactory, auditory, lens), is dependent on a series of reciprocal interactions between the epithelium and the stroma. The key pathways are Wnt/β-catenin, Eda/NF-κB, Fgf, Hedgehog, and Tgfβ/Bmp signaling [reviewed in (Biggs and Mikkola, 2014; Sennett and Rendl, 2012)]. Current understanding places widespread epidermal Wnt ligands at the top of the chain of interaction events. These epidermal Wnt ligands activate

Wnt/β-catenin signaling in the upper dermis which together with other, yet unknown dermal signals induces an epidermal response. This epidermal response includes β-catenin and leads to the localized formation of hair placodes [reviewed in (Hardy, 1992; Millar, 2002)]. Contemporaneously, beneath the epithelial placode the dermal condensate forms, which is marked e.g., by *Sox2* and *Tbx18* expression (Clavel et al., 2012; Grisanti et al., 2012).

For a long time, the early cellular and molecular events leading to dermal condensate formation remained elusive. However, a number of recent publications have now cast light on the formation of dermal condensates. Dermal condensate precursor cells start acquiring their molecular fate before they undergo directional migration and physically cluster and condense under the emerging hair placodes. This molecular fate includes the upregulation of Wnt/β-catenin signaling without which dermal condensates fail to form. While dermal condensate precursors pass through a highly proliferative stage, subsequent cell cycle exit is an early hallmark of dermal condensate formation. Moreover, without placode-derived FGF20 dermal condensates fail to form and even early condensate precursors are absent (Biggs et al., 2018; Gupta et al., 2019; Mok et al., 2019).

Our knowledge has also significantly advanced when it comes to the early cell fate choices within the hair placode. Ouspenskaia, Matos, and colleagues investigated how closely spaced progenitors in the placode can commit to differential fates (Matos et al., 2020; Ouspenskaia et al., 2016). Early placode cells undergo asymmetric divisions perpendicular to the basement membrane. As a result of these asymmetric divisions, Wnt signaling becomes restricted to basal daughter cells, while suprabasal daughter cells acquire the ability to respond to Hedgehog signaling. Ligands for both pathways are produced in the basal cells. Subsequently, basal cells exit the cell cycle and *Sox9*$^{+}$ suprabasal cells take over the production of new hair follicle-fated cells. Early on they produce *Sox9*$^{+}$ stem cells, while later they switch to the production of short-lived progenitors that differentiate into the hair lineages (Ouspenskaia et al., 2016). The early partitioning of Wnt signaling is one of the key steps in acquiring the differential fate. Intriguingly, basal placode cells produce both Wnt ligands as well as Wnt inhibitors. They place Wnt ligands basally to ensure their own Wnt activation as well as Wnt activation of the underlying mesenchyme. In contrast, Wnt inhibitors are placed apically to achieve Wnt restriction in suprabasal placode cells (Matos et al., 2020).

Once established, hair placode and dermal condensate consolidate a complex signaling network that promotes the hair follicle maturation and reinforces lateral inhibition on the neighboring epidermis. The newly formed hair follicle will undergo a stereotypical developmental program including the stages of hair germ (E15.5) and hair peg (E16.5-E17.5). Finally, upon engulfment of dermal papilla cells, the bulbous hair peg state is reached (E18.5). This process eventually leads to the mature hair follicle [reviewed in (Paus et al., 1999; Saxena et al., 2019; Schmidt-Ullrich and Paus, 2005)]. Markers such as *Sox9*, *Lgr6*, and *Lrig1* are initially co-expressed in the placode, but become increasingly compartmentalized as the follicle matures – thus setting the foundation for stem cell heterogeneity in the adult hair follicle (Jensen et al., 2009; Nowak

et al., 2008; Snippert et al., 2010b). Apart from the developing hair follicle, *Sox9*$^{+}$ cells also give rise to sebaceous gland precursors (Nowak et al., 2008).

In recent years, the understanding of the molecular pathways underlying the process of hair follicle development has improved tremendously. However, one of the remaining questions concerns how the molecular information gets translated into coordinated cellular behavior that ultimately drives hair follicle morphogenesis. A study by Ahtiainen et al. concluded that directional cell migration, but not placodal proliferation, is the cellular mechanism driving primary hair placode formation. They observe that the inhibition of actin remodeling suppresses placode formation, whereas the stimulation of the Eda and Wnt pathway increases cell motility and the number of cells committed to placodal fate. Intriguingly, cell fate choices and morphogenic events seem to be controlled by similar pathways, which might provide a convenient framework for the coordination of the two processes (Ahtiainen et al., 2014).

Since hair follicles do not develop in isolation, two questions arise: (a) How are hair follicles aligned with the body axis, and (b) how are hair follicles within a tissue patterned so that they are evenly spaced throughout the skin? While the alignment with the body axis seems to be instructed by a single pathway – the planar cell polarity pathway – (Cetera et al., 2017), the patterning process is more complex and controversial.

One line of evidence supports the existence of a reaction-diffusion mechanism based on WNTs, FGFs, and BMPs that determines placode size and placode spacing (Glover et al., 2017; Närhi et al., 2008; Noramly and Morgan, 1998; Sick et al., 2006). This mechanism would be in line with the chemical basis of morphogenesis proposed by Turing in the 1950s that relies on long-range inhibitors and short-range activators (Turing, 1952). Such a molecular pre-pattern would then direct the formation of mesenchymal aggregations. An alternative line of evidence supports a model that is also very reminiscent of the Turing model but rather relies on mechanical cues. This model suggests that in the first step, mesenchymal aggregates form as a result of cellular contractility which serves as a local activator and substrate stiffness which serves as a long-range inhibitor. These regularly spaced mesenchymal aggregates would then induce changes in epithelial gene expression via the mechanosensitive activation of β-catenin (Shyer et al., 2017).

Glover et al. succeeded in showing that the potential for both patterning mechanisms co-exists in developing mouse skin. He describes that under normal circumstances, the epidermal pre-pattern drives mesenchymal aggregations, but if such a pre-pattern is missing, self-organized aggregation can still happen in mesenchymal cells. It thus seems like patterning mechanisms follow a hierarchy (Glover et al., 2017).

1.6 NON-EPIDERMAL SKIN COMPARTMENTS

While the previous sections focused mostly on the epidermal compartment of the skin, it is important to keep in mind that the epidermis is just one of the skin layers. The underlying layers called dermis and hypodermis are also crucial for skin functionality. In addition to serving as a

scaffold, the dermis and hypodermis with all their components play an essential role in creating the cellular and molecular microenvironment that is needed for maintaining a fully functional epidermal barrier and hair coat.

1.6.1 Mesenchymal cells in the dermis and hypodermis

Adult homeostasis

Fibroblasts are the major cellular component of the dermis. Despite the fact that the presence of fibroblasts is imperative for tissue development and maintenance, they have long been regarded as mere scaffold cells. They have often even been thought of unfavorably for their detrimental role in tissue fibrosis where they deposit an excessive amount of extracellular matrix (ECM) [reviewed in (Sennett and Rendl, 2015)]. However, their crucial role in providing structural as well as molecular support is increasingly recognized.

One of the major functions of fibroblasts is to maintain the structural integrity of the dermis. Besides depositing ECM, most notably type 1 and 3 collagens, proteoglycans, and elastin [reviewed in (Watt and Fujiwara, 2011)], fibroblasts also need to constantly sense the mechanical properties of the dermis, such as ECM stiffness, and remodel ECM if needed. The major pathways implicated in sensing and responding to mechanical stress are YAP/TAZ and FAK signaling (Calvo et al., 2013; Wong et al., 2012).

Fibroblasts also display functional specialization that is linked to their location, especially during development. Dermal fibroblasts can be divided into two groups (Figure 7). The upper, papillary dermis, which is characterized by high cell density, high proliferation rate, and active Wnt signaling, and the lower, reticular dermis with increased deposition of ECM and expression of immune cell-associated genes. While the reticular dermis mediates the first wave of dermal repair after injury, the papillary dermis cells are recruited later during wound re-epithelialization and are required for new hair formation (Driskell et al., 2013; Harper and Grove, 1979; Philippeos et al., 2018; Rinkevich et al., 2015; Rognoni et al., 2016). Interestingly, this clear spatial segregation between papillary and reticular dermis can be seen in late embryonic and early postnatal skin, but with progressing dermal maturation it gets gradually lost (Rognoni et al., 2018; Salzer et al., 2018).

While dermal fibroblasts mature, they also change their proliferative behavior. Initially, fibroblasts populate the dermis by proliferation. However, already during early postnatal development (around postnatal day 10) proliferation slows down to a low rate which is maintained throughout a lifetime. Increased proliferation can always be initiated following cutaneous wounding (Driskell et al., 2013; Rognoni et al., 2016; Ruchti et al., 1983). In parallel with the downregulation of fibroblast proliferation, ECM deposition gets upregulated because proliferation and ECM deposition form a negative feedback loop (Rognoni et al., 2018).

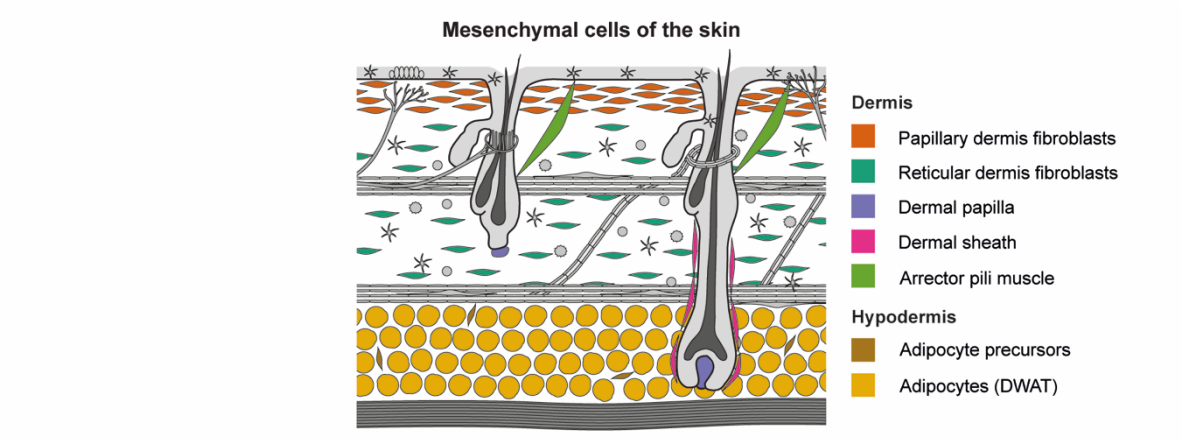

Figure 7: Mesenchymal cells of the skin [adapted from (Joost, 2019)].

Additional mesenchymal cells are associated with the hair follicle, such as cells in the arrector pili muscle, the dermal papilla, and the dermal sheath (Figure 7). The erector pili muscle is responsible for piloerection. The dermal papilla constitutes a mesenchymal signaling center and represents the adult counterpart to the embryonic dermal condensate. The dermal sheath surrounds the enlarged anagen hair follicle.

The dermal papilla is of utmost importance for hair cycling and the specification of hair follicle types (Driskell et al., 2009; Jahoda et al., 1984; Mesa et al., 2015b; Rompolas et al., 2012). In line with this, dermal papillae display considerable heterogeneity both between hair cycle phases as well as between hair types. For example, the transcription factor *Blimp1* is dynamically downregulated during anagen (Lesko et al., 2013; Telerman et al., 2017). And *Crabp1* and *Sox2* can be used to tell apart postnatal dermal papillae of guard hair follicles (*Sox2*$^{+}$/*Crabp1*$^{-}$), awl/auchene hair follicles (*Sox2*$^{+}$/*Crabp1*$^{+}$), and zigzag hair follicles (*Sox2*$^{-}$/*Crabp1*$^{+}$) (Clavel et al., 2012; Rezza et al., 2016).

During homeostasis, dermal papillae can be repopulated by progeny from the so-called hair follicle dermal stem cell (hfDSC) pool. Dermal papilla repopulation by hfDSCs is an important feature which becomes impaired during aging, resulting in a gradual net loss of dermal papilla cells which eventually leads to progressive hair loss (Shin et al., 2020). These bipotent stem cells can both repopulate the DP and also regenerate the dermal sheath (Rahmani et al., 2014). The dermal sheath tightly wraps the anagen hair follicle – starting just below the bulge – and is separated from the keratinocytes of the ORS by the basement membrane. During catagen, the dermal sheath envelops the degenerating epithelial strand, and during telogen, only a few hfDSCs are left, which wrap the dermal papilla [reviewed in (Martino et al., 2020; Yang and Cotsarelis, 2010)].

Beneath the dermis, there is the so-called hypodermis layer (Figure 7). It contains the dermal white adipose tissue (DWAT), which is separated from the subcutaneous white adipose tissue (SWAT) by the striated panniculus carnosus muscle (PCM). Intradermal adipocytes fulfill a plethora of tasks such as storing energy, participating in long-distance signaling, and providing insulation [reviewed in (Driskell et al., 2014)]. Moreover, they crosstalk with anagen epidermis. Epidermis-derived SHH and Wnt ligands lead to a massive expansion of DWAT,

which creates space, as well as structural and signaling support for the newly generated anagen hair bulb (Donati et al., 2014; Rivera-Gonzalez et al., 2016; Zhang et al., 2016), and adipocyte-derived PDGF promotes hair growth (Festa et al., 2011).

Embryonic/postnatal development

The developmental origin of the dermis and hypodermis varies depending on the body site. While cranial dermis/hypodermis is mostly of neural crest origin, trunk dermis/hypodermis has its main origins in the lateral mesoderm (ventral skin) and paraxial mesoderm (dorsal skin), respectively [reviewed in (Driskell and Watt, 2015)].

In mice, developmental lineages of dermal fibroblasts have been best studied in dorsal skin, where there is a common mesenchymal progenitor at E12.5. Lineage commitment happens around E16.5 and from E18.5 onwards, dermal development can be subdivided into the upper and lower lineage. The upper dermal lineage gives rise to the arrector pili muscle, papillary fibroblasts, and the dermal condensate, which gives rise to the dermal papilla and dermal sheath. The lower dermal lineage produces adipocytes and reticular fibroblasts (Driskell et al., 2013; Grisanti et al., 2012). It has been suggested that dermal and subcutaneous adipose tissue develop independently of each other, even though unequivocal experimental proof is pending (Wojciechowicz et al., 2013).

The formation of the arrector pili muscle is prompted upon deposition of nephronectin to the underlying basement membrane by posterior bulge stem cells. This nephronectin-rich niche prompts integrin $\alpha 8\beta 1$-expressing mesenchymal cells to upregulate smooth muscle markers and give rise to arrector pili muscles (Fujiwara et al., 2011).

1.6.2 Innervation & neural crest-derived cells

Adult homeostasis

The skin is a highly sensitive organ with dense innervation both in the dermis and epidermis. The dense neuronal network consists of both efferent and afferent neurons, with efferent neurons carrying information from the central nervous system to the periphery and afferent neurons carrying information in the opposite direction.

To discriminate between different sensations, the skin contains a wide variety of sensory, afferent neuron subtypes: nociceptors for painful stimuli, pruriceptors for itchy stimuli, thermoreceptors for thermal stimuli, and low-threshold mechanoreceptors (LTMRs) for non-painful mechanical stimuli and touch sensation. LTMRs are furthermore associated with so-called mechanosensory end organs. The contribution of the different neuron subtypes and end organs differs between hairy and glabrous skin. Glabrous skin is specialized in high-accuracy discriminative touch and contains several mechanosensory end organs: touch domes that detect static touch and are integrated into the epidermis, Meissner corpuscles that detect movement across the skin and are placed just below the epidermis, Ruffini endings that detect stretch and locate to the dermis, and Pacinian corpuscles that detect high-frequency vibration and are

placed in the deep dermis. Hairy skin performs touch sensation with slightly lower spatial acuity and contains touch domes as well as mechanoreceptive lanceolate endings that wrap around the upper bulge of hair follicles (Figure 8). Furthermore, in both hairy and glabrous skin, free nerve endings of unmyelinated LTMRs penetrate the epidermis [reviewed in (Zimmerman et al., 2014)]. Intriguingly, even nowadays new sensory end organs are still being discovered. It was long believed that noxious stimuli directly activate nociceptive sensory nerve endings in the skin. Only very recently, a novel glio-neural end organ made up of specialized cutaneous Schwann cells was discovered that is loaced at the dermal-epidermal-border in the skin (Abdo et al., 2019).

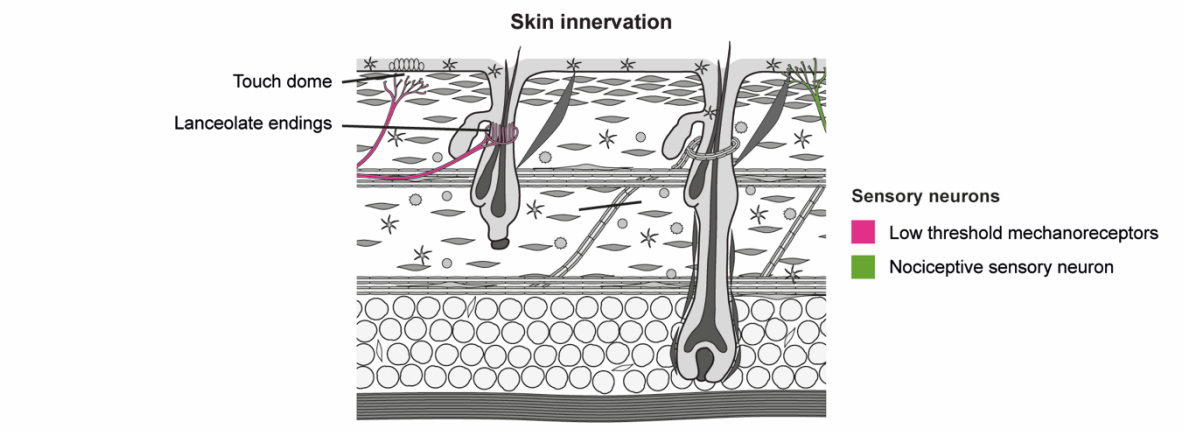

Figure 8: Sensory innervation of hairy skin [adapted from (Joost, 2019)].

Innervation also plays a special role in the hair follicle niche. It was shown that sensory nerves wrap around the upper hair follicle and secrete SHH, resulting in a subset of multipotent upper bulge stem cells expressing *Gli1*. Upon denervation, these peri-neural bulge cells lose their *Gli1*-expression. They can still contribute to tissue homeostasis and wound healing but seem to lose their ability to integrate long-term into newly generated wound epidermis. Thus, nerve-derived SHH may be of functional importance for hair follicle stem cells (Brownell et al., 2011).

The efferent neurons in the skin can be subdivided into motor neurons, which innervate the panniculus carnosus muscle via neuromuscular junctions (Holstege and Blok, 1989; Petruska et al., 2014; Theriault and Diamond, 1988), and sympathetic neurons. In the skin, sympathetic nerves control e.g., the activity of sweat glands as well as erection of hair follicles and nipples [reviewed in (Vetrugno et al., 2003)]. Interestingly, sympathetic nerve-derived norepinephrine can also activate hair follicle stem cells through ADRB2 receptors. Thus, cold leads to erected hair that traps air for insulation, as well as to a denser hair coat (Shwartz et al., 2020).

Peripheral neurons are also required for rapid wound healing. Upon injury, damaged peripheral neurons release high levels of neuromediators including IL1α, TNFα, NGF, and Substance P, which contribute to appropriate wound cellularity and to the inflammatory response required for successful wound healing [(Smith and Liu, 2002), and reviewed in (Scott et al., 2007)].

Other neural crest-derived components of the skin are Schwann cells, which envelop and support neurons, and melanocytes, the pigment-producing cells that in dorsal mouse skin are exclusively associated with hair follicles. During telogen, melanocyte stem cells reside in the bulge area (Nishimura et al., 2002). However, upon anagen entry, hair bulb-derived signals (e.g., EDN1) cooperate with dermal papilla-derived signals (e.g., KITL) to induce melanocyte relocation to the hair bulb as well as their proliferation and differentiation. Differentiated melanocytes transfer melanin granules to differentiating matrix cells, which in turn produce pigmented hair shafts. Upon progression to catagen, differentiated melanocytes degenerate while the stem cell pool in the bulge is maintained [reviewed in (Lin and Fisher, 2007; Tobin et al., 1999)].

There is a number of mechanisms resulting in the loss of melanocyte stem cells and concomitant hair greying: (a) age-related accumulation of DNA damage within melanocyte stem cells (Inomata et al., 2009), (b) loss of hair follicle stem cells due to age-related COL17A1 proteolysis, which results in secondary loss of melanocyte stem cells because they directly adhere to hair follicle stem cells (Matsumura et al., 2016; Tanimura et al., 2011), and (c) stress-related noradrenaline secretion by sympathetic nerves that induces rapid proliferation in usually quiescent melanocyte stem cells which ultimately leads to their differentiation, migration, and permanent depletion (Zhang et al., 2020).

Embryonic/postnatal development

During development, all cutaneous nerves, except for neural tube-derived motor neurons, are derived from the neural crest. Neural crest cells are transient cells that delaminate from the dorsal neuroepithelium and migrate along different routes to generate a variety of different cell types such as sensory neurons, sympathetic/parasympathetic neurons, glia of the peripheral nervous system (e.g., Schwann cells), and melanocytes. In the cranial compartment, they can even give rise to mesenchymal cell types [(Weston, 1970), and reviewed in (Le Douarin et al., 2004; Stifani, 2014)].

Heterogeneity of somatosensory neurons is a result of temporal waves of cell fate specification. The first wave of neurons is specified between E9.5 and E11.5 and is marked by NEUROG2. The second wave is specified between E10.5 and E13.5 and is marked by NEUROG1. Following their specification, they innervate the periphery (Frank and Sanes, 1991; Ma et al., 1999). Two models account for the development of the differential innervation pattern for each of the hair follicle types: (a) as both hair follicles and neurons are born in waves, the timing of arrival in the skin could determine which hair follicle type gets innervated by which neuronal subtype, or (b) each hair follicle type could express a specific set of molecular cues and each neuronal subtype a specific set of receptors for these cues. Most likely, it is a combination of the two strategies that ensures appropriate innervation of hair follicles [reviewed in (Jenkins and Lumpkin, 2017)].

How exactly the specialized mechanosensory end organs are generated is still under investigation. Mechanoreceptive lanceolate endings have been shown to be recruited to the hair

follicle postnatally through EGFL6-deposition by upper bulge stem cells (Cheng et al., 2018). Also, the developmental origin of touch-dome associated Merkel cells has been an area of intensive research. While they were originally believed to be of neural-crest origin, it is now well established that Merkel cells are of epidermal origin (Doucet et al., 2013; Morrison et al., 2009; Ostrowski et al., 2015; Szeder et al., 2003).

The sympathetic neurons innervating the arrector pili muscle are formed during embryogenesis, but then remain undifferentiated and quiescent for quite some time. Shortly after the arrector pili muscle has matured (approximately postnatal day 6-8; P6-8), the sympathetic nerves also differentiate (approximately P5-P11). Hair follicle-derived SHH as well as arrector pili muscle-derived NRTN seem to be involved in this process (Furlan et al., 2016; Shwartz et al., 2020).

Melanocytes were long thought to originate exclusively from neural crest-derived melanocyte precursors that delaminate from the dorsal neural tube, migrate between the superficial ectoderm and dermomyotome [reviewed in (Erickson, 1993)], and populate the epidermis around E13.5 (Yoshida et al., 1996). However, recent evidence demonstrates that melanocytes can also be derived from Schwann cell precursors that are located adjacent to nascent nerves (Adameyko et al., 2009). Another interesting feature is that melanocyte precursors initially populate interfollicular epidermis as well as hair follicles, but during postnatal development, only hair follicle-homed melanocyte stem cells persist (Hirobe and Enami, 2019).

1.6.3 Vascularization

Adult homeostasis

The dermal vasculature consists of two major structures: the superficial vascular plexus at the height of the telogen bulge and the deep vascular plexus that is located at the border of the dermis and hypodermis (Figure 9). The deep plexus contains larger vessels and is connected to the superficial plexus via vertically oriented vessels. The epidermis itself is avascular so it relies on nutrients obtained through diffusion from closely associated dermal capillaries [reviewed in (Barbieri et al., 2014)].

Additionally, there is a major venous plexus (termed ‘venule annulus’) wrapped around the upper bulge (Figure 9). Interestingly, the location of the venule annulus coincides with that of the perifollicular neural niche. This close association seems to be linked to the same secreted factor, namely EGFL6, that is involved in the recruitment of both nerves and vessels to that region. Interestingly, the venule annulus persists after denervation and even forms in de novo reconstituted hair follicles before their re-innervation, suggesting that the perivascular niche is of functional importance (Xiao et al., 2013).

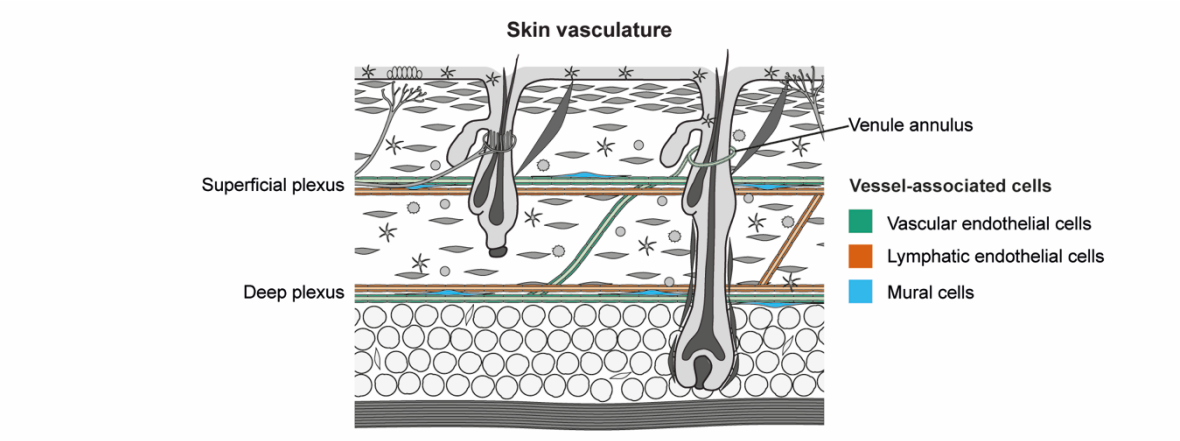

Figure 9: Skin vasculature [adapted from (Joost, 2019)].

In addition to trophic support and thermoregulation, cutaneous vasculature also seems to regulate hair cycling. Anagen induces substantial angiogenesis while catagen induces apoptosis of endothelial cells lining the vessels. Furthermore, pharmacological blocking of angiogenesis led to delayed anagen development, and inhibition of the pro-angiogenic factor VEGFA led to a delay in hair cycling as well as smaller hair follicles (Mecklenburg et al., 2000; Yano et al., 2001). A recent study uncovered more details on the crosstalk between hair follicle stem cells and cutaneous blood vessels. Vessels secrete factors such as BMP4 to maintain hair follicle stem cell quiescence, while the *Runx1* transcription factor in activated hair follicle stem cells mediates the expression and secretion of molecules involved in vascular remodeling (Li et al., 2019).

Blood vessels do not only consist of endothelial cells which line the vessel lumen, but they also contain mural cells, i.e., pericytes and vascular smooth muscle cells. While pericytes cover capillaries and venules, vascular smooth muscle cells surround arterioles. Pericytes and vascular smooth muscle cells are difficult to tell apart as the commonly applied markers are not specific. However, they can be reliably discriminated in adult tissues by the fact that pericytes share the basement membrane with endothelial cells while vascular smooth muscle cells reside outside of the basement membrane [reviewed in (Armulik et al., 2011; Nehls and Drenckhahn, 1993)].

While vascular smooth muscle cells fulfill the well-defined function of adjusting blood vessel volume and local blood pressure, pericyte functions are less well-defined and more diverse. They seem to (a) contribute to the deposition of ECM to the basement membrane, (b) sense physiological needs of the tissue including angiogenic stimuli, (c) signal to endothelial cells to control their proliferation and differentiation, and (d) integrate signals along the vessel length as they contact numerous endothelial cells [reviewed in (Gerhardt and Betsholtz, 2003)].

Besides the vascular network, the skin also contains a lymphatic network consisting of a superficial and a deep plexus (Figure 9) [reviewed in (Skobe and Detmar, 2000)]. Traditionally, lymphatic vessels were primarily linked to their function in regulating fluid homeostasis, macromolecule drainage in tissues, and immune cell trafficking [reviewed in (Schulte-Merker et al., 2011)]. However, recently they have also been implicated in the organ-specific

promotion of homeostasis [reviewed in (Wong et al., 2018)]. As it has been recently revealed that lymphatic vessels closely associate with hair follicles and get remodeled during the hair cycle (Gur-Cohen et al., 2019; Peña-Jimenez et al., 2019), it is compelling to assume that lymphatic capillaries could influence hair cycling e.g., by trafficking immune cells to the hair follicle at the appropriate phase of the hair cycle. As disruption of the lymphatic vasculature results in asynchronous cycling (Gur-Cohen et al., 2019), it is furthermore conceivable that lymphatic vasculature could redistribute signaling molecules within the tissue to achieve synchronization of hair cycling.

Embryonic/postnatal development

The vascular network in the skin is mostly formed by angiogenesis, i.e., the formation of new vessels from pre-existing vessels via sprouting or vessel splitting [reviewed in (Detmar, 2000). Tissues in need of vascularization release the major angiogenic inducer VEGFA which can be sensed by surrounding vessels [reviewed in (Ribatti et al., 2000)]. As a consequence, a single endothelial cell will become a so-called tip cell with extraordinary VEGFA-susceptibility thanks to VEGF receptor enrichment in their filopodia. This tip cell also starts expressing matrix metalloproteinases to degrade the vascular basement membrane that usually prevents endothelial cell migration. It furthermore expresses the Notch ligand DLL4 to prevent neighboring endothelial cells (so-called stalk cells) from responding to VEGFA and thus, to prevent the vessel from falling apart by excessive sprouting (Gerhardt et al., 2003; Ruhrberg et al., 2002). Notably, angiogenesis is a highly complex process and involves many more signaling molecules including other VEGF family members, PDGF family members, BMPs, PGF, TGFβ1, ECM components, and molecules better known from axon guidance (Semaphorins, Netrins, Neuropillins, Slit proteins) [(Cao et al., 2002; Detmar, 2000; Luttun et al., 2002; Newman et al., 2011), and reviewed in (David et al., 2009; Goumans and Mummery, 2000)].

Interestingly, for proper maturation of arterial vessels, they need to align to nerves. Nerve-derived chemokines serve as a guidance cue for sprouting angiogenesis and also endothelial cell-derived JUN-B seems to be involved in neurovascular alignment (Li et al., 2013; Mukouyama et al., 2002; Yoshitomi et al., 2017). Furthermore, for full vascular functionality and integrity, angiogenesis has to be followed by vascular remodeling, i.e., the recruitment of mural cells (Levéen et al., 1994; Soriano, 1994) (Levéen et al., 1994; Soriano, 1994).

There is a long-standing controversy as to whether endothelial tubes initially form without pericyte coverage and only mature and stabilize fully through subsequent acquisition of pericytes (Benjamin et al., 1998), or if pericytes are present much earlier in actively sprouting vessels to support various aspects of angiogenesis (Amselgruber et al., 1999; Reynolds et al., 2000). Most likely, the answer may look different in different angiogenic contexts.

Traditionally, lymphatic vessels were believed to form by sprouting from embryonic veins. However, it has now been shown that both venous and non-venous progenitors contribute to the formation of the dermal lymphatic network [(Martinez-Corral et al., 2015), and reviewed

in (Betterman and Harvey, 2018)]. The nature of the non-venous progenitors remains elusive though.

Notably, blood vessels have been reported to already start approaching nascent hair follicles at E14.5. By E16.5 they form a ring around the developing hair follicles (Xiao et al., 2013). Lymphatic vasculature is present at the time of hair follicle specification but only start approaching developing hair follicles during late embryogenesis (Peña-Jimenez et al., 2019).

1.6.4 Immune cells

Adult homeostasis

Skin as the body's primary environmental interface is constantly exposed to potential pathogen invasions and thus contains a dense network of immune cells that guard the skin against pathogens (Figure 10). Immune cells are categorized into the myeloid lineage containing monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, and megakaryocyte, and the lymphoid lineage containing T cells, B cells, natural killer cells, and innate lymphoid cells. The prevalence of the different immune cell subtypes depends on the body site, the skin layer, the proximity to hair follicles, and the hair cycle stage [reviewed in (Rahmani et al., 2020)].

The dermal immune cell compartment is dominated by mononuclear phagocytes, i.e., monocytes, macrophages, and dermal dendritic cells (DCs). For a long time, they have been difficult to tell apart and it has only been in the past decade that an antibody panel as well as detailed functional characterization became available (Tamoutounour et al., 2013).

Monocytes are best known in the context of inflammation when they extravasate in large numbers and differentiate into macrophages and inflammatory DCs. However, they also patrol extravascular peripheral tissue during homeostasis; most likely to promote peripheral tolerance (Jakubzick et al., 2013).

While macrophages in some tissues act as professional antigen-presenting cells, the major task of dermal macrophages lies in their contribution to tissue homeostasis through scavenging of cellular breakdown products and invading microorganisms (Tamoutounour et al., 2013).

The major antigen-presenting cells of the skin are classical DCs residing in the dermis. Both the $CD103^+$ and the $CD11b^+$ subset excel at migration to skin-draining lymph nodes and activation of T cells via MHC-bound peptides. Another subset is called inflammatory DCs or monocyte-derived DCs. They possess strong T cell stimulatory capacity but lack migratory abilities. This makes them well suited to induce T cells directly in the skin (Tamoutounour et al., 2013). The last dendritic cell subtype is called plasmacytoid DC. They are specialized in producing large amounts of type I interferons upon viral infections (Nakano et al., 2001; Siegal et al., 1999).

Dermis-resident mast cells can activate innate and adaptive immunity and act as effector cells exacerbating the development of allergic or autoimmune diseases [reviewed in (Morita et al., 2016)].

Dermis furthermore contains various T cell subsets. $CD4^+$ and $CD8^+$ resident memory T cells provide long-term local protection against infection. $FOXP3^+$ $CD4^+$ regulatory T cells regulate and suppress other immune cells. Lastly, dermal γδ T cells contribute to neutrophil recruitment through IL17 secretion [reviewed in (Rahmani et al., 2020)].

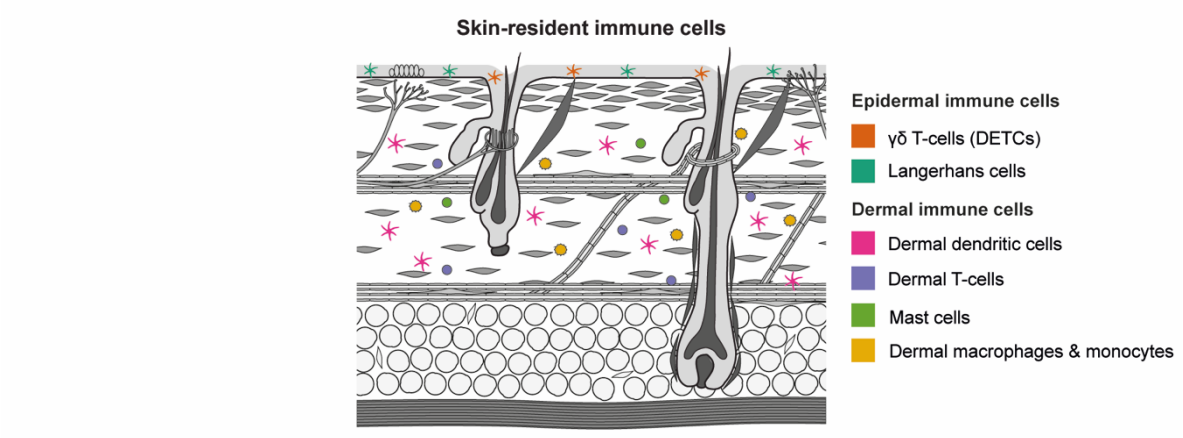

Figure 10: Skin-resident immune cells [adapted from (Joost, 2019)].

The immune composition of the epidermis is much simpler. There is a substantial number of Langerhans cells and dendritic epidermal T cells (DETCs) and low numbers of resident memory T cells. While cell bodies of the DETCs are placed in the basal layer of the IFE, the majority of Langerhans cells are located in the suprabasal layers, even though rare basal cells have also been described. With their numerous dendrites both Langerhans cells and DETCs scan the surrounding basal and suprabasal keratinocytes [reviewed in (Lombardi et al., 1993; Rahmani et al., 2020)]. A recent study revealed that Langerhans cell and DETC distribution follows an actively maintained tiling pattern. Moreover, they even adjust their abundance in response to altered basal keratinocyte density (Park et al., 2021).

Dendritic Langerhans cells are professional antigen-presenting cells that reside in the epidermis and monitor for foreign antigens. Once they captured antigens, they migrate to skin-draining lymph nodes to activate T cells [(Romani et al., 1989), and reviewed (Ginhoux and Merad, 2010; Kaplan, 2017)]. Keratinocytes are able to share mRNAs and proteins with Langerhans cells through direct intercellular material exchange. This represents a possible mechanism contributing to peripheral tolerance (Su and Igyártó, 2019).

DETCs are tissue-resident and show a dendritic phenotype; both features being highly untypical for lymphocytes [reviewed in (Gentek et al., 2018a; Thelen and Witherden, 2020)]. They also display a restricted T cell repertoire suggesting that they are only looking for predictable moieties of tissue damage (Asarnow et al., 1988). Furthermore, they function as cytokine secretors during the early stages of inflammation and tissue dysregulation [reviewed in (Hayday, 2009)].

While the previous paragraphs focused on the traditional functions of immune cells in cellular defense, the following paragraphs will showcase some non-canonical immune cell functions in the skin. In recent years, it has been increasingly recognized that immune cells can generate discrete milieus that favor processes such as proliferation, differentiation, and quiescence. As such, immune cells have proven themselves indispensable for hair cycling and barrier maintenance [reviewed in (Rahmani et al., 2020)].

Regulatory T cells localize close to the hair follicle bulge and facilitate hair cycle initiation through the expression of the Notch ligand JAG1 which promotes proliferation and differentiation of hair follicle stem cells (Ali et al., 2017). Regulatory T cells are furthermore important in the context of epidermal repair. They facilitate the contribution of hair follicle keratinocytes to the wound epithelium by preventing an overexuberant Th17 and neutrophil response (Mathur et al., 2019). Interestingly, infundibular keratinocytes express CCL20 ligand to keep regulatory T cells in the perifollicular space (Scharschmidt et al., 2017). Hair follicle keratinocytes furthermore ensure that skin-resident memory T cells are actively kept in the skin through the expression of IL7 and IL15 (Adachi et al., 2015).

Perifollicular mast cells contribute to anagen induction via secretory products – the exact nature of which remains elusive (Paus et al., 1994). Perifollicular macrophages maintain telogen via secretion of Oncostatin M, but they can also regulate anagen entry via coordinated apoptosis and concomitant release of WNT7B and WNT10A. Moreover, they promote catagen via FGF5 secretion (Castellana et al., 2014; Suzuki et al., 1998; Wang et al., 2019).

Also, γδ T cells are critical regulators of skin homeostasis and regeneration. The general absence of γδ T cells results in hair cycling defects (Kloepper et al., 2013). It has furthermore been shown that epidermal DETCs are critical for regulation of cutaneous inflammation, epidermal homeostasis, and wound repair and that they protect against epidermal tumors; their most important mediator is IL13 (Dalessandri et al., 2016; Girardi et al., 2002; Jameson et al., 2002). Dermal γδ T cells have been shown to promote wound-induced hair follicle neogenesis via Wnt activation in wound fibroblasts (Gay et al., 2013).

Taking together all these examples of close collaboration between immune cells and epidermal cells, the hair follicle immune system is emerging as a great system to uncover key principles of immune-mediated regeneration [reviewed in (Rahmani et al., 2020)].

Notably, keratinocytes themselves can also actively contribute to the immunological defense of the skin. Equipped with different pattern-recognition receptors, they can sense pathogen-associated molecular patterns and react by secreting cytokines, chemokines, as well as antimicrobial peptides [(Lebre et al., 2007; Miller and Modlin, 2007), and reviewed in (Tan et al., 2015)].

Embryonic/postnatal development

During embryogenic development, immune cells are successively produced at distinct hematopoietic sites [reviewed in (Orkin and Zon, 2008)]. Primitive hematopoiesis is initiated

in the extra-embryonic yolk sac around E7.0 and generates erythrocytes, macrophages, mast cells, and possibly even progenitors with lymphoid potential (Gentek et al., 2018a; Moore and Metcalf, 1970; Palacios and Imhof, 1993). Starting from E8.5 a new wave of hematopoietic progenitors is generated within the embryo proper; first in the paraaortic splanchnopleure region, then in the AGM region (aorta, gonads, mesonephrons) (Bertrand et al., 2005; Medvinsky and Dzierzak, 1996). Around E10.5 hematopoietic progenitors from the yolk sac and AGM region colonize the fetal liver, thymus, and spleen. After E11.5 fetal liver serves as the major hematopoietic organ. It contains mature hematopoietic stem cells (HSCs) and generates all hematopoietic lineages (Hoeffel et al., 2012; Kumaravelu et al., 2003; Naito et al., 1990). Ultimately, hematopoiesis in the bone marrow commences around birth [reviewed in (Orkin and Zon, 2008)].

Tissue-resident macrophages originate from so-called erythro-myeloid progenitors (EMPs). Early EMPs give rise to a first wave of yolk sac-derived macrophages, which have been reported to populate the whole embryo starting from E9.5 (Mass et al., 2016). Late EMPs relocate to the fetal liver and give rise to a second wave of fetal monocytes. These fetal liver-derived monocytes can differentiate into tissue-resident macrophages that are able to self-renew into adulthood (Gomez Perdiguero et al., 2015; Hoeffel et al., 2015; Mass et al., 2016).

Mast cell development follows a similar pattern, where a first wave of yolk sac-derived mast cells progenitors is later largely replaced by definite hematopoietic stem cell-derived mast cells. Similar to macrophages, mast cells self-maintain in the tissue, i.e., they replenish from local sources rather than relying on bone marrow-derived, circulatory cells (Gentek et al., 2018a). Mast cells have been reported to populate the dermis from around E14.5 (Gentek et al., 2018a; Hayashi et al., 1985).

Information on embryonic development of DCs is sparse. The only exception is the development of epidermis-resident Langerhans cells which is well-described and will be discussed below. For other skin-resident DCs, only the adult situation is well-described where dermal DCs arise from circulating bone marrow-derived macrophage and DC progenitors (MDPs). Dermal DCs have a short half-life and do not self-renew within the tissue (Fogg et al., 2006).

Langerhans cells are very different from other skin-resident DCs when it comes to development. They are derived from embryonic progenitors, which seed the developing skin before birth and are maintained as local precursors allowing for self-renewal throughout a lifetime independent from circulating precursors. They display a dual developmental origin where a minority of Langerhans cells originate from early yolk sac-derived macrophages while the majority of Langerhans cells arise from fetal liver-derived monocytes (late embryogenesis) (Chorro et al., 2009; Hoeffel et al., 2012). Langerhans precursors have been reported to enter the epidermis around E16.5 (Weiss and Zelickson, 1975) but they do not acquire a mature Langerhans signature including CD207 and MHC II-molecules until birth (Tripp et al., 2004).

DETCs share a lot of developmental features with Langerhans cells rather than with conventional T cells. DETCs are established from fetal thymic progenitors that home to the skin around E16.5 (Elbe et al., 1989). However, the majority of DETCs only appear after birth and their numbers keep steadily increasing during early postnatal development (Romani et al., 1986). Similar to Langerhans cells, homeostatic maintenance of DETCs is achieved through clonal expansion of tissue-resident precursors and independent from circulating hematopoietic cells (Gentek et al., 2018a). And indeed, depletion of DETCs in adults is not followed by their re-appearance (Aberer et al., 1986). The developmental origin of DETCs is an area of ongoing research, but it seems like they might arise from yolk sac-derived progenitors with lymphoid potential (Böiers et al., 2013; Gentek et al., 2018b; Palacios and Imhof, 1993; Yoshimoto et al., 2012).

The precursors of all other T cells arise in the bone marrow and subsequently relocate to the thymus, where they differentiate. A constant replenishment by circulatory T cells is kept up throughout a lifetime [reviewed in (Havran and Allison, 1988)].

1.7 CUTANEOUS WOUND HEALING

As the skin is the body's most important protective barrier, any injury poses a serious threat, and the barrier has to be rapidly and efficiently restored. To this aim, mammals have developed a complex cellular program that regenerates the skin barrier [reviewed in (Martin, 1997)].

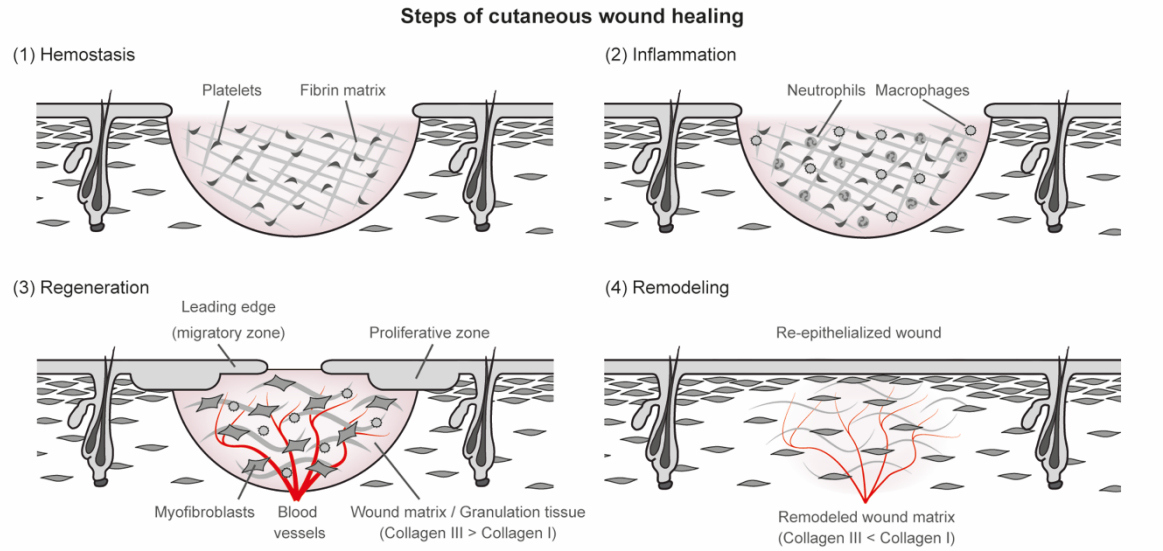

Figure 11: Characteristic stages of cutaneous wound healing [adjusted from (Joost, 2019)].

Cutaneous wound healing follows a four-step program (Figure 11): (1) The first step is hemostasis. Vessels are constricted in order to reduce blood flow and a fibrin- and fibronectin-rich clot is formed from platelets and coagulation cascade factors to provisionally close the wound. The fibrin clot also serves as a migration scaffold for other cells and as a source for chemotactic cues. (2) Within hours the inflammatory stage commences where inflammatory cells are recruited. It starts with a wave of neutrophils that kill bacteria and degrade damaged matrix proteins and is followed by a wave of macrophages that remove tissue debris and destroy remaining neutrophils. (3) Next, wound healing enters the regenerative phase where re-

epithelialization is initiated. The delay of re-epithelialization can be explained by the fact that first, a provisional wound matrix has to be formed on which the keratinocytes can crawl, and secondly, integrins and actin filaments have to be re-arranged in order to allow for migration of keratinocytes. The regenerative stage also includes the formation of granulation tissue, a wound-specific connective tissue. Additionally, angiogenesis begins, and myofibroblasts try to pull together the wound edges. (4) The final remodeling stage aims at the restoration of the epidermis, dermis, and extracellular matrix in order to regain some of the original functionality of the tissue. Due to the complexity, this stage can last for months (reviewed in (Arwert et al., 2012; Martin, 1997)).

Role of epidermal cells

The process of re-epithelialization, that has been mentioned above, is most important for restoring the skin barrier. It describes the formation of new epidermal tissue on top of the wound matrix. Fibroblasts and immune cells within the granulation tissue activate keratinocytes in the immediate wound-surrounding via EGFs, FGFs, HGFs, KGFs, PPARs, TGFβ, acetylcholine, catecholamines, among others to trigger their proliferation and migration to the wound site. These signals show a large degree of redundancy indicative of the important nature of re-epithelialization [reviewed in (Werner et al., 2007)].

Intriguingly, it is not only keratinocytes from the interfollicular epidermis that get recruited and contribute to wound healing. Upon full-thickness wounding also cells from wound-surrounding hair follicles (first 2-3 rows) can join the coordinated stream of epithelial cells moving towards the wound. Multiple cell populations from the hair follicle such as *Lgr5*$^{+}$, *Lgr6*$^{+}$, *Krt14*$^{+}$, *Krt15*$^{+}$, *Lrig1*$^{+}$ cells have been reported to contribute (Aragona et al., 2017; Füllgrabe et al., 2015; Ito et al., 2005; Kasper et al., 2012). The contribution of hair follicle keratinocytes might not be essential to wound healing, but it can clearly accelerate wound closure (Langton et al., 2008). Depending on their original location in the IFE or hair follicle, different stem cell populations can also show distinct temporal dynamics. While cells from the upper hair follicle can reach the wound front as early as 24 hours after injury, bulge-derived cells show a visible delay. Also, the long-term contribution differs between the different contributing stem cell populations. It is a matter of ongoing debate though, if those differences result from diverging stem cell potential or different initial numbers of cells arriving at the wound front (Page et al., 2013).

Recent advances in intravital imaging of mouse skin have allowed skin researchers to investigate how skin tissue can orchestrate the cellular mechanisms needed to re-epithelialize a wound. The skin epithelium around a wound site can be divided into an actively proliferating ring, a non-proliferative, migratory wound front – also known as leading edge –, and a tissue expansion zone in-between, with coexisting proliferation and migration. Cells transition out of the proliferating ring through directional division and then start an active migration towards the wound (Park et al., 2017). In order to successfully migrate on the provisional wound matrix, keratinocytes upregulate genes involved in extracellular matrix remodeling and cell adhesion,

such as integrin α5β1, proteases and matrix metalloproteinases [reviewed in (Martin, 1997; Rognoni and Watt, 2018)].

During wound healing, epidermal stem cells become much more plastic than during homeostatic settings. While their contribution pattern is usually very restricted, the injury setting allows them to cross compartment-borders in order to help with re-epithelialization. Epidermal stem cells have even been reported to change their identity during wound healing. Interfollicular stem cells at the wound edge transiently induce the transcription factors *Sox9* and *Tcf3* that are typical for hair follicle stem cell identity. In contrast, mobilized hair follicle stem cells permanently induce transcription factors linked to interfollicular stem cell identity such as *Klf5* and *Ap2γ* (Ge et al., 2017). Another long-standing question has been if post-mitotic, differentiated cells without contact to the basement membrane can de-differentiate and contribute to epidermal wound repair. Concerning this matter, Donati et al. recently reported that *Gata6*$^{+}$ cells, which usually reside in the sebaceous duct as suprabasal cells, upon wounding can transition back to the basal epidermal layer and contribute to wound healing. Progeny of *Gata6*$^{+}$ cells that contributes to wound healing loses the typical sebaceous duct markers including *Gata6* (Donati et al., 2017).

Despite all these recent advancements of our understanding, which have uncovered unexpected plasticity including lineage infidelity and de-differentiation, the exact molecular changes that epithelial cells undergo when migrating towards the wound site have been difficult to dissect. It is insufficiently understood, how epidermal stem cells can break their homeostatic compartment boundaries and how much the different stem cell populations converge while they adopt a common wound-induced migration state. Moreover, it remains to be answered, how much of their original identity keratinocytes keep after integration into the newly formed wound epidermis.

Role of non-epidermal cells

While much of the research has been focused on the epidermal component of wound healing, the dermal component of wound healing is just as important. Many fibroblasts in the vicinity of the wound become rapidly activated, most importantly by TGFβ, and turn into *α smooth muscle actin*-expressing myofibroblasts with an altered behavior and transcriptional program. As their major function is wound contraction, they have a transient nature and undergo apoptosis at the end of the wound healing process (Driskell et al., 2013; Dulauroy et al., 2012; Plikus et al., 2017).

Distinct fibroblast populations show differential recruitment and contribution to wound healing (Correa-Gallegos et al., 2019; Driskell et al., 2013; Rinkevich et al., 2015; Rognoni et al., 2016). Fibroblasts from the reticular dermis and fascia are the major source of myofibroblasts, and papillary fibroblasts enter the wound contemporaneously with re-epithelialization and exclusively relocate to the upper dermis. Dermal papilla cells do not seem to contribute to wound repair (Kaushal et al., 2015). Intriguingly, depletion or manipulation of specific

mesenchymal subsets in the dermis can lead to a significant reduction of tissue fibrosis or scar formation upon tissue injury (Dulauroy et al., 2012; Rinkevich et al., 2015).

Adipocytes and preadipocytes have also been shown to be crucial for successful wound healing as defects in adipogenesis result in impaired fibroblast recruitment and dermal reconstruction. Within hours after injury, wound edge adipocytes start proliferating and dedifferentiating and repopulate the wound bed as myofibroblasts. Additionally, adipocyte lipolysis releases fatty acids into the wound which are essential for the robust recruitment of inflammatory macrophages (Schmidt and Horsley, 2013; Shook et al., 2020).

Mesenchymal cell types also support nerve sprouting in the wound bed. During deep skin injuries, cutaneous sensory axons and receptors are destroyed, while the neuronal cell bodies in the dorsal root ganglia persist. Myofibroblasts aid re-innervation by secreting neurotrophins as well as by producing ECM components such as laminin that are known to promote neuronal outgrowth [(Constantinou et al., 1994; Palazzo et al., 2012; Rivas et al., 1992), and reviewed in (Sidgwick and Bayat, 2012)]. Unfortunately, itching and pain are common sequalae of healed wounds [reviewed in (Iannone et al., 2019)].

Scar formation

Even though many cell types collaborate to heal the wound, skin injury often results in scar tissue rather than a fully functional reconstitution. Scar tissue is characterized by an excess of ECM, as well as the lack of hair follicles, sweat glands, and cutaneous fat. The main producers of scar tissue are myofibroblasts that, given their rapid proliferation and ECM-producing abilities, are able to produce a lot of tissue in a short time. But this comes at the expense of tissue quality. It is thus important that during the final phase of wound healing, (a) ECM is remodeled – type 3 collagen is replaced with type 1 collagen, and elastin, which is important for skin elasticity, re-appears –, and that (b) myofibroblasts undergo apoptosis. Inappropriate delay of myofibroblast apoptosis may be one of the driving factors underlying excessive scaring (reviewed in (Lebonvallet et al., 2018; Plikus et al., 2017)).

In addition to the described sequence wound healing events, a number of other phenomena can be observed. Firstly, the hair follicles around the wound site go into anagen much earlier than the rest of the skin. This results for example from macrophages that are recruited to the wound site and then activate hair follicle stem cells, leading to telogen-to-anagen transition around the wound (Wang et al., 2017). Secondly, wounds are re-epithelialized fastest if wounding occurs during the anagen stage. This might be caused by alterations in the epithelial, endothelial, and inflammatory cells in the skin during the hair cycle, or by the fact that genes beneficial for wound healing and genes upregulated in anagen are actually correlated (Ansell et al., 2011). Thirdly, while small excisional wounds $<1cm^2$ are typically repaired by forming scar tissue devoid of epidermal appendages and fat, large wounds ($> 1cm^2$) in mice can regenerate *de novo* hair follicles and adipocytes in their center. Strikingly, both wound-induced hair neogenesis (WIHN) as well as *de novo* fat regeneration employ the reactivation of developmental programs (Ito et al., 2007; Plikus et al., 2017). Among the cell populations and signaling events that

positively impact WIHN, papillary fibroblasts, macrophages, dermal γδ T cells, FGFs, and WNTs have gained most attention (Driskell et al., 2013; Gay et al., 2013; Ito et al., 2007; Osaka et al., 2007). There are still numerous open questions about the intriguing WIHN effect, one of them being why WIHN is only ever observed in large wounds and even there only in the center of the wound.

1.8 COMPARISON OF DIFFERENT MOUSE BODY SITES

In mice, the most commonly studied body areas are dorsal skin, ear skin, paw skin, and tail skin. They differ e.g., in epidermal thickness, contribution of adnexal structures, and immune composition. This makes the distinct regions particularly well suited for different kinds of research questions.

Tail and paw skin display a much thicker IFE and thus the different stratified layers are much more pronounced than in dorsal and ear skin which usually only present with 2 or 3 cell layers.

The typically referred to four hair follicle types (guard, awl, auchene, and zigzag) are representative for pelage hair from the trunk [reviewed in (Sundberg et al., 2005)].

While melanocytes of dorsal skin are strictly restricted to the hair follicles, in the ear, ventral paw, and the tail they can also reside in the IFE [reviewed in (Sarin and Artandi, 2007)].

Paw skin presents with a special composition of adnexal structures. It is made up of (a) the plantar paw skin without any skin appendages i.e., neither hair follicles nor any glands, and (b) the ventral footpads, which lack hair follicles but are the only anatomical region in the mouse that contains eccrine sweat glands [reviewed in (Scudamore et al., 2014)].

Tail skin displays a very characteristic patterning. In tail, hair follicles are arranged in groups of three. The IFE around those hair follicle openings is called interscale and follows the regular epidermal differentiation program (so-called orthokeratotic differentiation). The remaining IFE is called scale and displays a particular differentiation program called parakeratotic differentiation. Most importantly, parakeratotic IFE lacks the granular layer and nuclei are retained in the cornified layer [reviewed in (Gomez et al., 2013)].

Ear skin has gained massive popularity for live imaging as (i) it is very thin, (ii) hair follicles grow at a steep angle and thus are located very close to the IFE surface, and (iii) it is rather stable against movements caused by breathing [reviewed in (Pineda et al., 2015)].

Additionally, differences in the immune composition of the skin at different body sites have been observed. For example, numbers of epidermis-resident Langerhans cells and DETCs were reported to be significantly reduced in paw and tail skin. Also, dermis-resident immune cells showed tissue-specific enrichment i.e., macrophages were enriched in ear and paw skin, mast cells were enriched in ear skin, and dermal T cells were enriched in dorsal skin (Tong et al., 2015).

1.9 COMPARISON OF HUMAN AND MOUSE SKIN

The laboratory mouse is the most widely used model organism for biomedical research. This is a result of their simple and economical maintenance, fast reproduction, as well as the fact that a wide range of transgenic models and mouse-specific reagents and tools are available. But of course, mice are much smaller than humans, have a significantly shorter life expectancy, and do not efficiently reproduce all aspects of human tissue homeostasis and diseases. The skin of large animal models such as the Duroc pig is more similar to human skin, but these animals are expensive to maintain, require manipulation training, and lack the extensive characterization and tools that are available for mice. Thus, mice are still highly relevant for studying skin biology. It is important though, to keep in mind the differences between human and mouse skin, which will be summarized below [reviewed in (Wong et al., 2011)].

The overall anatomical structure of human and mouse skin is comparable. The skin of both species contains hypodermis, dermis, as well as the typical four layers of the stratifying epidermis. However, the mouse epidermis of most body areas is much thinner than the human epidermis (2-3 layers across <25µm vs. 5-10 layers across >100µm). Mechanical properties also show clear differences: while the mouse dermis is thin, compliant, and loose, the human dermis is thick, relatively stiff, and adheres to the underlying tissue. Furthermore, the mouse epidermal-dermal border is flat, while it is undulating in humans and forms so-called rete ridges. While the complete mouse trunk contains a subcutaneous muscle layer (panniculus carnosus muscle), humans only retain rudiments of that muscle in the neck region. Moreover, human melanocytes are located in the hair follicles and IFE, while they are mostly restricted to hair follicles in mice. Eccrine sweat glands cover almost the entire human body surface, while in the mouse they are restricted to the footpads [reviewed in (Gudjonsson et al., 2007; Wong et al., 2011; Zomer and Trentin, 2018)].

The typical hair cycle phases of telogen, anagen, and catagen are shared between humans and mice. However, while mouse hair cycles only last weeks and are – at least in the initial postnatal hair cycles – synchronized, human hair cycles can last up to years and they are highly asynchronous [reviewed in (Braun-Falco et al., 2000; Müller-Röver et al., 2001)]. Moreover, mice display a high density of hair follicles on most body sites, while human skin presents typically with sparse hair follicles. Also, in mouse skin, the previous hair shaft is retained while the new hair shaft is growing, whereas in human skin the old hair shaft is lost in an additional hair cycle phase called exogen. Another specialty of human skin is the differentiation between unpigmented vellus hair and pigmented terminal hair and the androgen-mediated switch between them [reviewed in (Wong et al., 2011; Zomer and Trentin, 2018)].

The four phases of wound healing described in section *1.7 Cutaneous wound healing* are shared between human and mice. However, wound contraction, which is an important feature of mouse skin, is absent in human skin. Multiple experimental models have been developed that employ humanized mice or that prevent wound contraction in mice in order to increase the translational value of wound healing studies performed in mice [reviewed in (Wong et al., 2011; Zomer and Trentin, 2018)].

It has also been recognized that mice display a strong gender difference in skin phenotype. In male mice, the dermis is much thicker and firmer than its female counterpart, while epidermis and hypodermis are thicker in females. Reports of similar gender differences in humans are missing to date [reviewed in (Wong et al., 2011; Zomer and Trentin, 2018)].

Importantly, also immunology differs between human and mouse skin. One of the most clear-cut differences is the absence of DETCs in the human epidermis. Furthermore, human skin contains many more memory T cells and far fewer naïve T cells. This mostly reflects the fact that laboratory mice are kept under carefully controlled specific pathogen-free conditions and thus encounter significantly fewer pathogens throughout their lifetime [reviewed in (Ho and Kupper, 2019; Rahmani et al., 2020)].

2 METHODS

2.1 SINGLE-CELL RNA SEQUENCING

For the longest time, stem cell research has relied on techniques that study single markers or individual parameters. In a typical workflow, expression of individual marker genes would be used to define cell populations, which would then be analyzed quantitatively or qualitatively using qPCR, as well as antibody-based FACS-analysis and immunohistochemistry. However, this approach is intrinsically biased and relies heavily on *a priori* knowledge. Furthermore, it assumes a high degree of homogeneity among the analyzed cell populations.

Thanks to the recent revolution in the field of transcriptomics, which now allows us to study individual transcriptomes of a large number of cells, we have come to realize the cellular heterogeneity displayed by complex tissues and developmental processes. In the following section, major advances in the experimental and computational approaches for single-cell RNA sequencing (scRNA-seq) will be presented and discussed.

Library preparation:

High throughput single-cell transcriptomics was pioneered in 2009 by Tang and colleagues (Tang et al., 2009). Ever since, a great variety of methods for the generation of single-cell transcriptome libraries has been developed. Each of these approaches had to deal with the major challenge for obtaining scRNA-seq libraries, i.e., the miniscule amounts of RNA present in each cell (10-30pg). Solutions to prevent sample loss include minimized reaction spaces, reduced washing steps, and pooling of tagged cDNA from many different cells [reviewed in (Chen et al., 2018a)].

The general workflow of scRNA-seq library preparation consists of: (1) capturing single cells, (2) lysing single cells in separate reaction environments, (3) reverse transcription of the released mRNA, second strand synthesis and contemporaneous addition of cell-specific identifiers, (4) pooling of tagged cDNAs and cDNA amplification, (5) fragmentation, and (6) sequencing (Figure 12) [reviewed in (Chen et al., 2018a)]. However, despite the shared general workflow, substantial differences between the specifics of each step can be found in the different experimental protocols.

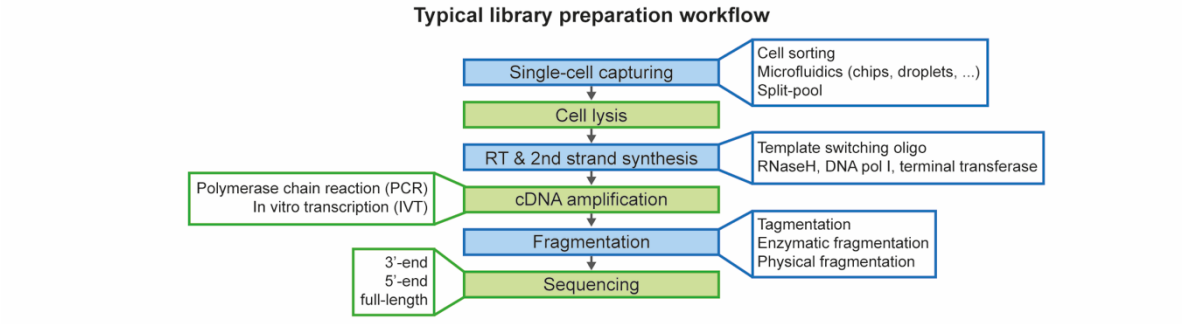

Figure 12: Typical steps during the library preparation for scRNA-seq [adapted from (Joost, 2019)].

While initial studies still relied on manual cell picking (Islam et al., 2011; Ramsköld et al., 2012; Tang et al., 2009), the first milestone for increased capturing efficiency was reached with the introduction of microfluidic systems, such as the Fluidigm C1 chip (Islam et al., 2014), and with the adaptation of library preparation protocols for FACS-isolated cells in 384 well plates (Jaitin et al., 2014). Throughput was increased even more with the introduction of droplet-based microfluidic systems, in which single droplets function as reaction environments (Klein et al., 2015; Macosko et al., 2015). The Chromium system by 10x Genomics is a commercialized droplet-based platform, that has gained widespread popularity for its ease of use. Similar throughput is achieved by nanowell-based methods (Gierahn et al., 2017). Currently, split-pool barcoding achieves the highest cell numbers (hundreds of thousands of transcriptomes from one experiment) (Rosenberg et al., 2018).

Once cells are captured and lysed, mRNA needs to be transcribed into cDNA. Most scRNA-seq protocols initiate reverse transcription with oligo-dT primers that selectively target mRNA. Second strand synthesis is usually performed with the help of template switching oligos. This allows for the incorporation of additional sequences such as primer binding sites and sequencing adapters into the second strand of the cDNA. Alternatively, RNase H, DNA polymerase I, or terminal transferase can be used. Due to the low amount of cDNA that is retrieved from each single-cell reaction, cDNA has to be strongly amplified. This is most commonly done by PCR using the primer binding sites that were introduced during second strand synthesis. However, PCR is prone to amplification bias, i.e., different cDNA molecules might get amplified with different efficiency. To counteract amplification bias, many protocols employ so-called unique molecular identifiers (UMIs). UMIs are short randomized oligonucleotide sequences that are introduced into each cDNA molecule during reverse transcription or second strand synthesis. They make it possible to link each sequencing read to the original cDNA molecule, and thus alleviate the effect of amplification bias and sequencing errors (Islam et al., 2014). Another added sequence is the cellular barcode, which is usually introduced during reverse transcription, second strand synthesis, or after amplification. Finally, barcoded cDNA libraries of all cells can be pooled and processed for sequencing. Next-generation sequencing technologies require fragmentation of libraries and the addition of adapter sequences. Fragmentation is usually done enzymatically, physically, or with the help of transposomes (also called tagmentation) [reviewed in (Chen et al., 2018a)].

While the majority of scRNA-seq protocols produce cDNA libraries covering the full-length of mRNAs, most methods only sequence either the 5'-end or the 3'end of each fragment to reduce costs. This still suffices to determine the transcript abundance of all mRNA molecules expressed in a particular cell. However, it prevents more in-depth analysis such as the detection of splice isoforms or single nucleotide polymorphisms. Another disadvantage is that libraries are sparser than those derived from protocols with full-length coverage; full-length coverage in this case means that cDNA libraries are still fragmented, but that the full length of the fragments is sequenced. A major disadvantage of previous full-length protocols such as Smart-seq and Smart-seq2 has been their lack of UMI sequences (Picelli et al., 2014; Ramsköld et al.,

2012). However, the recently introduced Smart-seq3 protocol now combines full-length gene coverage with UMIs (Hagemann-Jensen et al., 2020).

Sequencing of scRNA-seq libraries is usually performed on Illumina sequencing platforms, which results in high throughput and cost-effective short reads (Bentley et al., 2008). However, there are also sequencing platforms (e.g., Pacific Biosciences) for non-fragmented, full-length cDNA (Sharon et al., 2013), as well as sequencing platforms (e.g., Oxford Nanopore) that can directly sequence mRNA molecules (Oikonomopoulos et al., 2016).

After sequencing, the produced reads have to be demultiplexed based on their cellular barcodes, they have to be aligned to the reference genome, and in the case of UMI-based protocols, data can be demultiplexed even more to obtain absolute count data.

While much of the method development efforts over the years have focused on increasing throughput while reducing the sequencing costs per cell, we have now come to a point where datasets of several hundred-thousand cells are not uncommon anymore. It is thus time to reconsider the optimal ratio of sampling depth and cell number as well as to determine if cellular heterogeneity is better captured by sampling many cells from few individuals or fewer cells from many individuals [(Bhaduri et al., 2018), and reviewed in (Tanay and Regev, 2017)].

In **Paper I and IV**, the microfluidics-based STRT-C1 protocol was utilized to analyze hundreds of cells. In **Paper II and III**, the droplet-based 10x Genomics Chromium system was utilized to analyze thousands of cells.

Data analysis:

Along with the advances in scRNA-seq protocols, computational methods had to learn the characteristics of scRNA-seq data (such as extraordinary sparsity) and find ways to accommodate them. The toolbox for scRNA-seq analysis has been expanding ever since the introduction of scRNA-seq. With the increased popularity and availability of scRNA-seq, efforts also went into facilitating scRNA-seq analysis by developing integrated pipelines; especially Seurat (in the R programming language) and Scanpy (in the Python programming language) are worth highlighting (Butler et al., 2018; Wolf et al., 2018). Furthermore, best-practice guides have become available to sensitize for common mistakes and to aid in decision-making during the data analysis workflow (Figure 13) (Luecken and Theis, 2019).

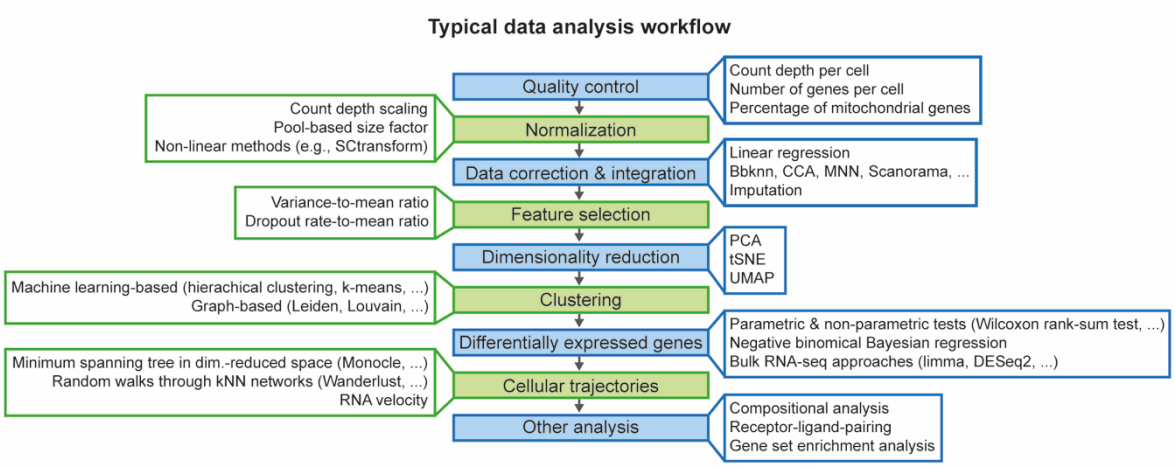

Figure 13: Typical steps during the analysis of scRNA-seq data.

Preprocessing is the initial step of scRNA-seq analysis and usually includes quality control, normalization, data correction and integration, feature selection, and dimensionality reduction [reviewed in (Luecken and Theis, 2019; Wagner et al., 2016)]. During quality control, low-quality cells are typically excluded based on count depth, number of detected genes, and/or percentage of mitochondrial reads. This aims at removing low-quality cells as well as cell doublets. Normalization tries to remove cell-to-cell variation caused by differences in sequencing depth, capture efficiency, or other technical confounders. Most commonly, count depth scaling is used to obtain correct relative gene expression abundances between cells. However, this method is based on the assumption that all cells within a dataset originally contained an equal amount of mRNA molecules, which is rather unlikely in heterogeneous datasets. Pool-based size factor normalization does not share this assumption, and thus might be better suited for heterogenous scRNA-seq datasets (Lun et al., 2016). Lately, also non-linear normalization techniques such as SCtransform have been suggested (Hafemeister and Satija, 2019). In the next step, normalized data is usually $\log_e(x+1)$-transformed which reduces the skewness of the data and better approximates the assumption of many downstream analysis methods that the data is normally distributed.

At this point, normalized data might still contain unwanted variability caused by technical covariates (e.g., batch) as well as biological covariates (e.g., cell cycle). Fitting linear regression models with the covariates as predictors is a common approach to remove unwanted variability that might hide the biological information that is of interest. The challenge though is to remove covariates while preserving the key biological differences. More recently, a number of advanced integration methods have been developed that even allow for the integration of multimodal datasets (Butler et al., 2018; Haghverdi et al., 2018; Hie et al., 2019; Polański et al., 2020). Furthermore, imputation methods have been developed to deal with dropout events. Imputation methods use co-expression patterns across cells to obtain expression estimates that can be used for downstream analysis instead of the measured zero-inflated expression levels (van Dijk et al., 2018; Eraslan et al., 2019).

In **Paper I**, we did not use any data normalization. In **Paper II**, data was normalized using the pool-based size factor. In **Paper III**, data was normalized using count depth scaling.

Additionally, linear regression and BBKNN (batch balanced k-nearest neighbors) were performed to correct for batch effects. In **Paper IV**, data was normalized using count depth scaling.

Feature selection is the next critical step in the scRNA-seq analysis pipeline. It aims at selecting the most informative genes that explain most of the variability within the dataset. Typically, 1000-5000 highly variable genes (HVGs) are selected based on their variance-to-mean ratio. An alternative approach selects those genes that have the highest dropout rate given their mean expression level (Andrews and Hemberg, 2019). To further reduce the complexity of the dataset before clustering, dimensionality reduction using principal component analysis (PCA) is commonly performed. This reduces calculation time in subsequent analysis steps but also alleviates problems linked to zero-inflation and transcriptional variation. Dimensionality reduction is also performed to allow for the visualization of highly complex datasets in 2D space. The most common choices for visualization are t-distributed stochastic neighbor embedding (t-SNE) (van der Maaten and Hinton, 2008) and uniform manifold approximation and projection (UMAP) (McInnes et al., 2018). Furthermore, diffusion maps are oftentimes used when modeling cellular trajectories, as they more faithfully capture gradual identity changes (Haghverdi et al., 2015).

In **Paper I** and **IV**, we identified HVGs based on the variance-to-mean ratio and exclusively used t-SNE to visualize the data and as input for trajectory reconstruction. In **Paper II** and **III** visualization was done with UMAP and t-SNE in parallel, while trajectory reconstruction was based on diffusion maps. HVGs were identified based on the variance-to-mean-ratio (**Paper II**) or dropout-to-mean-ratio (**Paper III**), respectively.

To identify distinct cell populations, it is common practice to perform unsupervised clustering on single-cell transcriptomes. There are two major categories of clustering algorithms. The first one represents machine learning algorithms such as hierarchical clustering, affinity propagation, and k-means clustering. They are well established but assume roughly equally sized clusters. The second category includes graph-based algorithms such as Louvain and Leiden that focus on community detection within networks. Their major advantages are that they scale well with large datasets and they excel at identifying cell populations of different sizes and densities. However, they rely on effective representation of the data in a k-nearest neighbor (kNN) graph [reviewed in (Andrews and Hemberg, 2018)]. To further characterize identified cell populations, differentially expressed genes for each cell population are identified. This is classically done using parametric or nonparametric statistical tests such as the t-test or the Wilcoxon rank-sum test. However, it can also be done using alternative approaches, such as e.g. negative binomial Bayesian regression approaches or approaches originally developed for bulk RNA-seq such as limma and DESeq2 [reviewed in (Luecken and Theis, 2019)].

In **Paper I**, we employed affinity propagation. In **Paper II**, we employed affinity propagation and validated clustering results with Louvain clustering. In **Paper III**, a combination of hierarchical clustering and Leiden clustering was used. In **Paper IV**, k-means clustering was

performed. DEG identification was based on a negative binomial Bayesian regression approach (**Paper I** and **IV**) or the Wilcoxon rank-sum test (**Paper II** and **III**), respectively.

While the identification of discrete cell populations is very useful to understand tissue heterogeneity, many processes in living organisms are continuous rather than discrete. scRNA-seq data is particularly well suited to analyze gradual identity changes such as developmental processes, homeostatic differentiation processes, as well as injury-induced regenerative processes. While developmental and regenerative processes might require sampling on multiple consecutive time points, homeostatic differentiation processes usually can be captured with a single sampling time point. This is because those processes most often are not synchronized so that cells from different stages of the process co-exist within tissue and scRNA-seq is able to resolve them. Multiple computational tools have been developed to infer and model cellular trajectories. They have in common that they order single-cell transcriptomes along a unidirectional or branching axis (so-called pseudotime), which can subsequently be used to model gene expression along the pseudotime and to identify regulatory networks and transcription factors critical for the process [reviewed in (Saelens et al., 2019; Wagner et al., 2016)]. Algorithms such as Monocle (Trapnell et al., 2014) construct a so-called minimum spanning tree in dimensionality-reduced space, while algorithms such as Wanderlust (Bendall et al., 2014) or diffusion pseudotime (Haghverdi et al., 2016) perform random walks through kNN networks. The decision on an algorithm for trajectory inference should be based primarily on the dataset dimensions as well as the trajectory topology.

A major limitation of most lineage reconstruction methods is that they cannot assign directionality to the obtained cellular trajectories. Thus, RNA velocity, which was presented in 2018, represents a novel exciting tool that complements traditional lineage reconstruction methods by considering data from spliced and unspliced transcripts (Bergen et al., 2020; La Manno et al., 2018). RNA velocity is based on the notion, that expression of unspliced transcripts of gene X precedes the expression of spliced transcripts of that same gene X because it takes some time to splice newly transcribed transcripts. Thus, unspliced transcripts are assumed to reflect a later stage of the cell and they allow for the inference of directionality in differentiation processes.

Besides trajectory inference, scRNA-seq also provides the opportunity to study interactions between different cell types. This is commonly done by identifying potential receptor-ligand pairs between different cell populations (Cabello-Aguilar et al., 2020; Efremova et al., 2020).

In **Paper I** and **IV**, a modified version of Monocle was used to infer IFE differentiation (**Paper I**) as well as gradual identity changes of epidermal stem cells during cutaneous wound healing (**Paper IV**). In **Paper II** and **III**, we employ a combination of diffusion pseudotime and RNA velocity to model hair follicle matrix differentiation (**Paper II**) and differentiation of embryonic fibroblasts and keratinocytes (**Paper III**). Additionally, in **Paper II**, **III**, and **IV**, a receptor-ligand analysis was performed using a custom pipeline.

2.2 SPATIALLY RESOLVED TRANSCRIPTOMICS

To properly understand the complex network of diverse cell types and their functions within tissues and organs, both gene expression as well as spatial context, are of utmost importance. Only the combination of those two enables us to robustly assign cell types, understand where they are placed within a tissue, and unveil which other cells surround them. *In situ* stainings are also important to validate annotated cell populations i.e., ensuring that the computationally identified cell populations are correct and meaningful. During the last decade, a number of experimental and computational methods have been developed that allow us to link positional information with gene expression data. Some of the major methods as well as their limitations will be presented hereafter.

The roots of *in situ* transcriptomics lie in a method called *in situ* hybridization (ISH), which was pioneered by Gall and Pardue in 1969 (Gall and Pardue, 1969). Since then, radio-labeled probes have been replaced by fluorescent or chromogenic probes and a plethora of molecular techniques have been developed that significantly advanced the detection of mRNA *in situ* [reviewed in (Pichon et al., 2018)].

One of the major milestones in mRNA *in situ* detection was the development of single-molecule fluorescent ISH methods (smFISH) which came with a major increase in sensitivity and specificity. The key to success was to utilize much shorter probes for targeting the RNA of interest (10-50 nucleotides instead of kilobase-long probes) so that several probes can bind to the same mRNA molecule. Furthermore, the different smFISH methods have developed different signal amplification schemes. This leads to a significant increase in local signal strength compared to traditional FISH, which enables the detection of single mRNA molecules. Among the most popular amplification approaches are hybridization chain reaction (HCR), branched DNA (bDNA) amplification, and rolling circle amplification (RCA) (Figure 14) [reviewed in (Pichon et al., 2018)].

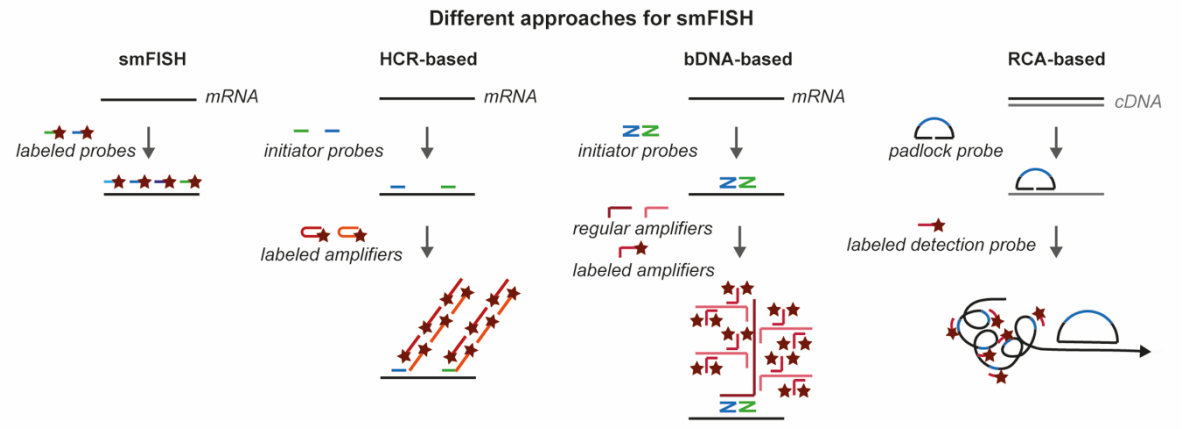

Figure 14: Different amplification strategies for single-molecule FISH (smFISH) methods. HCR: hybridization chain reaction. bDNA: branched DNA. RCA: rolling circle amplification.

In HCR, an initiator probe hybridizes to the RNA target and triggers the self-assembly of fluorescently-labeled RNA hairpin reporters (Choi et al., 2010).

In bDNA-based technologies, a number of self-complementary probes build a DNA tree with multiple levels of amplification, and only the final amplification oligonucleotide is fluorescently labeled (Kern et al., 1996; Player et al., 2001). This approach is commercialized through the RNAscope platform. Newer versions (RNAscope v2) combine this tree-approach with tyramide signal amplification that increases the local density of fluorophores even more (Wang et al., 2012).

RCA-based approaches are mostly based on so-called padlock probes i.e., oligonucleotides whose two end sequences bind to the reverse-transcribed target sequence and are joined together by high-fidelity DNA ligases. This results in a closed, single-stranded DNA circle that serves as the template for phi29 DNA polymerase-mediated RCA which results in a micrometer-sized bundle of DNA. As the amplified DNA contains detection sites for fluorescently-labeled detection oligos, a strong local fluorescent signal can be obtained from each RCA-product (Larsson et al., 2010; Nilsson et al., 1994). Notably, mismatches at the ligation site are not tolerated, which enables classical padlock probe-based assays to resolve single nucleotide polymorphisms (SNPs) and point mutations (Grundberg et al., 2013).

The most limiting step in padlock probe-based assays when it comes to sensitivity is the generation of cDNA by reverse transcription (RT). RT on fixed tissue sections is both inefficient and costly. Nevertheless, it is required because conventional DNA ligases show only minor activity on DNA/RNA hybrids such as the DNA padlock probe bound directly to the RNA target. Two recent methods managed to circumvent RT. clampFISH utilizes click-chemistry to ligate DNA probes after direct hybridization to RNA targets (Rouhanifard et al., 2019). SCRINSHOT takes advantage of the SplintR ligase which shows good activity on DNA/RNA hybrids (Sountoulidis et al., 2020). Moreover, those two methods increase the throughput through sequential detection rounds.

To increase multiplexing even more, seqFISH (Lubeck et al., 2014) employs combinatorial barcoding. Each targeted transcript is encoded by a unique color sequence that is read out over multiple imaging rounds. Using temporal barcoding, the number of resolvable targets scales with the number of fluorophores and the number of imaging rounds. However, to obtain good results, images of the subsequent rounds have to be reliably aligned to allow for faithful decoding of the transcript. Thus, the more detection rounds are included, the more likely it becomes that targets are misidentified. To counteract this drawback, error-correction schemes such as the Hamming distance-based error correction were incorporated in methods such as MERFISH (Chen et al., 2015).

The remaining challenges mostly revolve around auto-fluorescence, spatial crowding within cells, the large number of gene-specific probes that are required, and the necessity for complex algorithms for image analysis [reviewed in (Lein et al., 2017; Mayr et al., 2019)]. Approaches to overcome those challenges include clearing (Moffitt et al., 2016), expansion microscopy (Chen et al., 2016), and signal amplification (Shah et al., 2016).

In addition to the aforementioned smFISH methods, a number of *in situ* sequencing approaches have been developed.

In situ sequencing (ISS) was originally developed by the Nilsson lab and sequencing-by-ligation is performed either on a barcode in the padlock probe backbone or on a gap-filled sequence that is complementary to the target sequence (Ke et al., 2013). It is a powerful method, but due to the short sequencing read length (4 nucleotides) and the size of the RCA products, the number of targets is still limited.

BaristaSeq (Chen et al., 2018b) builds on ISS but read-length was increased to 15 nucleotides and instead of sequencing-by-ligation Illumina sequencing chemistry is used.

STARmap (Wang et al., 2018) avoids RT by utilizing a duplex consisting of a padlock probe and a primer binding right next to the padlock probe. It furthermore relies on hydrogel-tissue chemistry, clearing of lipids and proteins, and sequencing-by-ligation. STARmap has been successfully used to visualize up to 1000 genes within intact tissue sections.

FISSEQ (Lee et al., 2014) employs RCA without utilizing padlock probes. cDNA is cross-linked to its cellular environment, circularized, and then RCA is performed on the circularized cDNA fragments. Importantly, this makes FISSEQ unique from all the previously described methods because it presents a truly untargeted approach that does not rely on *a priori* chosen probes. However, spatial crowding within a cell prohibits the use of FISSEQ for whole transcriptome analysis.

Truly transcriptome-wide approaches include Geo-seq and spatial transcriptomics.

Geo-seq (Chen et al., 2017) combines laser capture microdissection of a small number of cells from a specified region in the tissue with scRNA-seq. It results in single-cell transcriptomes without losing all the spatial information, but it is very labor-intensive and requires prior knowledge to decide on the dissected region.

Spatial transcriptomics (Ståhl et al., 2016) instead, provides a truly unbiased approach. Thin tissue sections are placed on glass slides with immobilized capture beads containing oligo-dT primers. Each spot on the glass slide has a unique positional barcode sequence. Tissue is imaged to capture the morphological characteristics and subsequently cells are permeabilized so that mRNA is released and can hybridize to the capture beads. After reverse transcription, sequencing libraries are generated. Importantly, each of the obtained transcriptomes can be traced back to its original location within the tissue. A major limitation of the first generation of spatial transcriptomics is the spot size. Individual spots have a diameter of 100µm and thus capture tens of cells. Thus, while spatial transcriptomics is truly unbiased and transcriptome-wide it does not yet achieve single-cell resolution. However, the commercialized Visum technology (10x Genomics) already decreased spot diameter to 55µm and bead-based approaches such as Slide-seq (Rodriques et al., 2019) and HDST (Vickovic et al., 2019) as well as photolithography are promising developments that might allow for a continued increase in resolution [reviewed in (Larsson et al., 2021)].

In addition to this wealth of experimental approaches, there are also computational approaches that aim at reconstructing spatial data from scRNA-seq data with the help of ISH atlases (Achim et al., 2015; Halpern et al., 2017; Karaiskos et al., 2017; Satija et al., 2015). The atlases are used to generate spatial gene expression reference maps to which single-cell transcriptomes can be mapped based on gene expression profile matches. However, these approaches require the existence of ISH atlases. A novel approach named novoSpaRc aims to computationally infer spatial organization even in the absence of known expression patterns of landmark genes, however applicability for complex tissue architectures is still limited (Nitzan et al., 2019).

Lastly, it is important to put the sensitivity of the different methods into context. scRNA-seq covers the whole transcriptome and detects between 5 and 40 % of all transcribed mRNA molecules, depending on the sequencing depth and chosen technology. smFISH can at its best detect almost 100 % of mRNA molecules but only for a limited number of target genes. *In situ* barcode sequencing can capture about 5 % of transcribed mRNAs for an intermediate number of genes. And FISSEQ can in principle detect all cellular transcripts but suffers from very low sensitivity (approximately 0.01 %) [reviewed in (Lein et al., 2017)].

Taken together, methods for capturing spatial information alongside single-cell sequencing information, have greatly advanced in the past decade and have brought together the previously separated worlds of imaging and sequencing.

All papers included in this thesis build on the powerful combination of transcriptome-wide information from scRNA-seq and spatial information obtained from smFISH stainings. In **Paper I**, a bDNA-based smFISH method (RNAscope v1) was used to map identified cell populations back to the tissue using selected marker genes. In **Paper II, III, and IV**, the improved version of RNAscope (v2) was used that combines bDNA-based smFISH with TSA signal amplification.

2.3 LINEAGE TRACING MOUSE MODELS

One of the central questions in stem cell research is to understand to which lineages the offspring of a given stem or progenitor cell can contribute. Genetic lineage tracing models have been indispensable for answering this question for a wide variety of stem and progenitor cells.

The majority of lineage tracing models rely on the combined expression of a driver recombinase under a tissue- or cell-type specific promoter and a reporter gene (most often a fluorescent marker or β-galactosidase) under a ubiquitous promoter (usually the *Rosa26* locus (Soriano, 1999)). In the absence of the recombinase, expression of the reporter gene is prohibited by a STOP sequence. Only in the presence of the recombinase, recombination happens, and the STOP sequence is removed. Importantly, this leads to reporter gene expression not only in the recombined cell but also in all descendent cells; thus, the method is called lineage tracing (Figure 15).

Lineage tracing methods can be broadly divided into two. In constitutive lineage tracings, the driver recombinase is permanently expressed in a tissue and is activating expression of the

reporter gene whenever the tissue- or cell-type specific promoter becomes activated. In contrast, inducible lineage tracing is achieved with a modified driver recombinase, which requires an external stimulus (e.g., heat or a drug) to relocate to the nucleus where it can activate reporter gene expression. This allows researchers to mark cells at a very specific time point and thus facilitates more advanced experimental settings [reviewed in (Kretzschmar and Watt, 2012; McKenna and Gagnon, 2019)].

During the past two decades, the Cre-LoxP-system has been the most widely used lineage tracing system in mice [reviewed in (Sauer, 1998)]. Cre recombinase enables constitutive lineage tracing via removal of a STOP cassette that is flanked by two LoxP sites, while the modified CreER^{T2} allows for inducible lineage tracing that is initiated through the supply of tamoxifen (Feil et al., 1997).

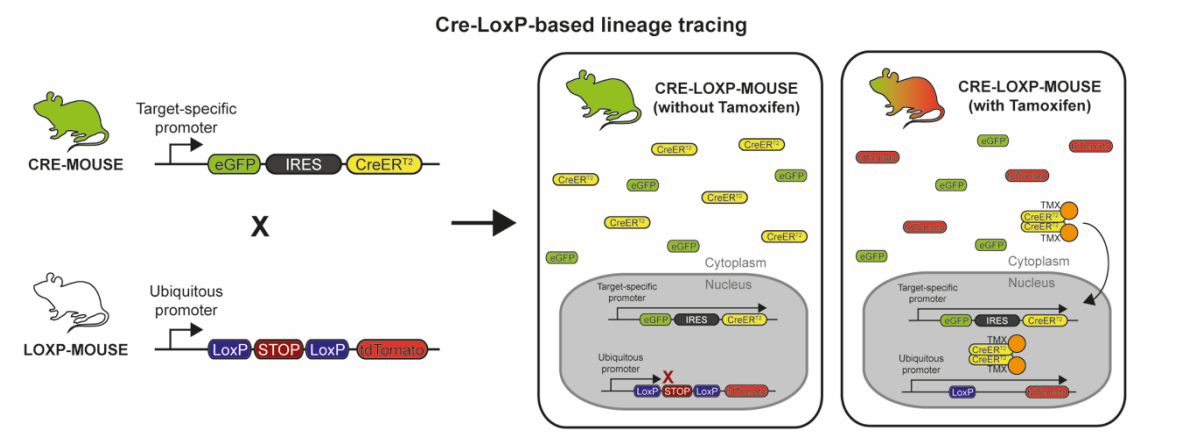

Figure 15: Exemplary inducible lineage tracing experiment as used in Paper III and IV. Inducible CreER^{T2} recombinase is expressed under a cell type-specific or tissue-specific promoter and accumulates in the cytoplasm. Only upon the addition of tamoxifen (TMX), it can relocate to the nucleus and achieve recombination. Recombination allows a reporter gene (in our case the fluorescent protein tdTomato) to be expressed under a ubiquitous promoter (in our case Rosa26).

When using drugs like tamoxifen to induce lineage tracing it is important to keep in mind that those agents themselves may cause side effects and alter the studied process or perturb homeostasis of the studied tissue. It is thus important to always include appropriate controls and to titrate the drug to the minimal dose that still achieves reliable labeling (Bhatia et al., 2010).

Lineage tracings have been refined in a number of ways. One of the most popular systems relies on the combinatorial expression of fluorescent proteins, which allows for the marking of multiple clonal populations (Livet et al., 2007). Lately, a novel set of single-cell genetic lineage tracing strategies have been developed that make it feasible to solve much more complex lineage trees than with traditional lineage tracings systems. These methods employ viral barcodes, the Polylox mouse model, or tracking of somatic mutations in combination with single-cell DNA sequencing [reviewed in (Kester and van Oudenaarden, 2018)]. Recently, a CRISPR/Cas9-based mouse line has been generated that allows for developmental barcoding (Kalhor et al., 2018). Most likely, in the soon future it will be combined with transcriptome-coupled single-cell readouts.

In **Paper III** and **Paper IV**, an inducible Cre-LoxP-based lineage tracing system was utilized with $CreER^{T2}$ recombinase and the highly sensitive reporter gene tdTomato. In **Paper III**, lineage tracing was used to explore the developmental contribution of a specific mesenchymal subpopulation (*Gata6*$^+$ fibroblasts). In **Paper IV**, specific epithelial stem cells (*Lgr5*$^+$ and *Lgr6*$^+$ keratinocytes, respectively) and their progeny were fluorescently labeled and FACS-sorted to allow for their molecular analysis with scRNA-seq.

3 KEY QUESTIONS OF THE APPENDED PAPERS

This thesis aimed to understand the molecular and cellular heterogeneity, the spatial organization, and the dynamic nature of mouse skin during adult tissue homeostasis, embryonic development, and tissue regeneration after injury.

Mammalian epidermis consists of the interfollicular epidermis that undergoes a stereotypic stratification program and the hair follicle which contains multiple compartments with distinct stem cell populations. In **Paper I**, we addressed the following key questions

- How heterogenous are epidermal cells?
- Which factors determine epidermal heterogeneity?
- Is epidermal heterogeneity better described by gradients or distinct compartments?
- How does the molecular program of epidermal stratification look like?
- What confers stem cell identity to previously identified epidermal stem cell populations?

Hair follicles undergo cyclical renewal that requires epidermal remodeling to achieve hair production, but also massive reconstruction of the stromal compartment to accommodate the enlarged hair follicles. In **Paper II**, we addressed the following key questions

- How is skin remodeled to accommodate hair growth?
- Is this process accomplished through the same cell types that maintain skin at rest, or does it require altered cellular states or even differentiation of new cell types in both epidermis and stroma?
- How do matrix cells achieve production of the inner hair follicle lineages?

In the embryo, epidermal stratification and hair follicle induction is preceded by maturation of the stromal compartment. In **Paper III**, we addressed the following key questions

- How heterogenous are dermal fibroblasts during early skin development?
- How do embryonic fibroblasts support skin maturation?
- Which microenvironmental changes occur before the epidermis stratifies and forms appendages?
- Is there molecular heterogeneity in the epidermis prior to hair follicle induction?

Re-epithelialization is the most crucial step during cutaneous wound healing as it re-establishes the epidermal barrier. In **Paper IV**, we addressed the following key questions

- Do progeny from different epithelial stem cell niches that are recruited to the wound site acquire a common transcriptional program?
- How do hair follicle cells adapt in order to become wound front and long-term wound epidermis progenitor cells?
- When and where during wound healing do the critical changes to cellular identity occur?

4 KEY RESULTS OF THE APPENDED PAPERS

The papers included in this thesis together draw a detailed molecular picture of mouse skin during adult tissue homeostasis (**Paper I** and **II**), embryonic development (**Paper III**), and tissue regeneration after injury (**Paper IV**). They reveal uncharted heterogeneity and allow us to explore how stem cell identity is shaped and how regenerative processes are orchestrated. In the following, I will present the major findings for each of the included studies.

4.1 PAPER I: MOLECULAR HETEROGENEITY OF MOUSE SKIN DURING REST (TISSUE HOMEOSTASIS)

New concepts and results: *First-ever scRNA-seq dataset of skin. Continuous rather than discrete signatures shape transcriptional heterogeneity in the epidermis. A very basal identity is what unites epidermal stem cells residing in different niches.*

This study represents the first whole-transcriptome study of skin at the single-cell level. Using the STRT-C1 platform, cells from the epidermal compartment of resting, mouse skin (telogen; 8 weeks of age) were analyzed. A major achievement of Paper I was to set up scRNA-seq for keratinocytes. By virtue of their physical properties, such as being very sticky, keratinocytes are inherently difficult targets for capturing and library preparation for scRNA-seq. Successful scRNA-seq led to 1 422 single-cell transcriptomes derived from 19 mice.

These cells could be assigned to 13 main populations reflecting heterogeneity within the interfollicular epidermis (5 populations), the upper hair follicle (3 populations), and the lower hair follicle (2 populations). Additionally, sebaceous gland cells and epidermis-resident immune cells, i.e., Langerhans and γδ T cells, were also captured. Further subclustering revealed additional, more subtle differences in gene expression and e.g. novel cell populations lining the sebaceous gland opening that could potentially play a critical role in protecting the hair follicle bulge against microorganisms (Figure 16A). Importantly clustering was performed unsupervised without taking into account any prior knowledge. This allowed for truly unbiased exploration of the epidermal transcriptional landscape. As the spatial context is lost during scRNA-seq a major effort was undertaken to map back every single population to a specific region within the skin with the help of protein and mRNA stainings (immunohistochemistry and smFISH, respectively).

Intriguingly, despite the multitude of subpopulations, more than 95 % of the heterogeneity among epidermal cells could be explained along two axes (Figure 16B). The first axis (termed ‘pseudotime’) reflects the epidermal differentiation program, which was derived from IFE cells. Surprisingly, pseudotime-dependent gene expression can also be observed in the hair follicle suggesting that the differentiation program is universal for almost all epidermal keratinocytes in resting skin. The second axis (termed ‘pseudospace’) captures the gradual gene expression changes along the proximal-distal axis of the hair follicle. Pseudospace also illustrates that at least on the transcriptional level, cell populations in the hair follicle are characterized by gradients rather than strict boundaries. This is also reflected in the observation

that cell populations located at the border of compartments often show overlapping gene signatures.

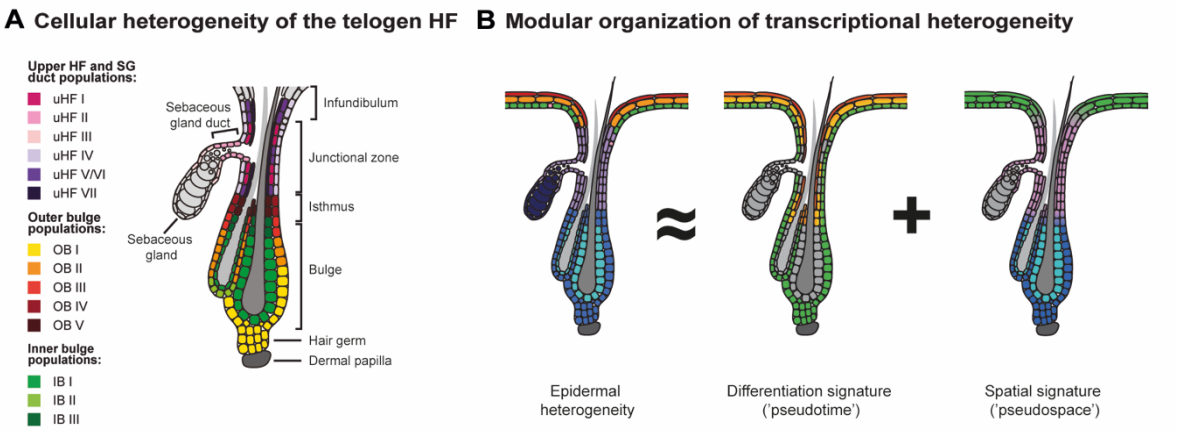

Figure 16: (A) Cellular heterogeneity among keratinocytes of the telogen hair follicle uncovered by scRNA-seq. HF: hair follicle. SG: sebaceous gland. uHF: upper hair follicle. OB: outer bulge. IB: inner bulge. (B) The majority of transcriptional heterogeneity among epidermal cells can be explained by a model containing only two predictors: pseudotime and pseudospace [adapted from (Joost et al., 2016)]

As stem cell markers such as *Cd34*, *Gli1*, *Lrig1*, *Lgr5*, *Lgr6*, and high *Krt14* have been widely adopted in the skin field, we wondered if there is a stem cell-unifying transcriptional signature that sets them apart from other basal keratinocytes not expressing any of those stem cell markers. It turned out that stem cell marker-expressing basal keratinocytes all share a very basal gene signature, while most of the variation between them can be ascribed to the spatial signature of the niche in which they reside. This supports the idea that any basal cell could be a stem cell and that stem cells should be defined by function, rather than by stem cell markers.

Taken together, the results of **Paper I** provide the most systematic and in-depth analysis of gene expression in the skin at the time of publication and paint a detailed picture of how cellular heterogeneity can be orchestrated in vivo to assure tissue homeostasis.

4.2 PAPER II: MOLECULAR HETEROGENEITY OF MOUSE SKIN DURING HAIR GROWTH (TISSUE HOMEOSTASIS)

New concepts and results: *Most comprehensive characterization of full-thickness anagen and telogen mouse skin to date. Companion layer – classically considered an inner hair follicle lineage – transcriptionally resembles outer hair follicle lineages. Evidence for transcriptionally uncommitted matrix progenitors. Modeling of gene expression changes during inner lineage differentiation.*

This study generated a systematic scRNA-seq-based molecular atlas of full-thickness skin during rest (telogen, 9 weeks of age) and growth (anagen, 5 weeks of age), which captures the process of hair growth. Epidermal and stromal cell types were included to represent the intricate interplay of multiple cell types that shapes the physiology of mouse skin as well as to study how stromal cell types help to accommodate hair growth.

Full-thickness mouse skin was dissociated, and single cells were randomly captured using the 10x Genomics Chromium system. Two datasets were generated (main dataset and validation dataset), each containing more than 5 000 cells. The following major cell classes were identified in the combined dataset: epidermal cells (including IFE cells, the permanent part of the hair follicle, and the cycling part of the hair follicle), fibroblasts and fibroblast-derived cells (dermal sheath and dermal papilla), vascular cells (vascular and lymphatic endothelial cells and vascular smooth muscle cells), immune cells (Langerhans cells, T cells, macrophages), neural crest-derived cells (Schwann cells and melanocytes), skeletal muscle cells, and red blood cells. Subclustering revealed more than 50 robust cell populations.

To understand how the skin both achieves and accommodates hair growth, we checked for the enrichment of cell types in anagen or telogen skin, respectively. The abundance of most cell populations was unchanged between anagen and telogen. However, secondary hair germ was exclusively found in telogen (as expected), while the anagen hair follicle, melanocytes, and some fibroblast subtypes showed substantial remodeling and enrichment in anagen. Some cell populations such as IFE cells and the permanent part of the hair follicle did not necessarily change in abundance, but increased proliferation and metabolic activity following telogen-to-anagen transition.

Next, we focused on the cycling part of the hair follicle – a highly heterogeneous structure. As the anagen hair follicle is traditionally described to be composed of inner and outer layers, we expected to find two major keratinocyte subgroups. Instead, we found three.

As it turned out, the outer layer cells could be divided into two transcriptionally highly distinct clusters of cells, that at least in the mature anagen follicle do not seem to transition to each other and that possess differential ability to signal with the stromal compartment. These two distinct groups could be annotated as basal outer layer cells (including two basal ORS clusters as well as the lower proximal cup) and suprabasal outer layer cells (including one suprabasal ORS cluster, mid-part companion layer, and upper companion layer). That the companion layer cells cluster with the outer lineages is highly surprising as the companion layer is classically considered one of the seven inner lineages of the anagen hair follicle.

The inner layers displayed a branching architecture with a central hub of matrix cells and three differentiating branches representing the IRS lineage, the Cortex/Cuticle lineages, and the Medulla lineage (Figure 17). RNA velocity as well as Markov modeling revealed that matrix cells are transcriptionally still uncommitted. Clear lineage commitment did only happen at the root of the branches. We furthermore uncovered that cells upon commitment contemporaneously exit the cell cycle and induce an intermediate molecular program, which ensures cell differentiation along a specific lineage. This intermediate molecular program encompasses genes that are neither found in progenitors nor in terminally differentiated cells.

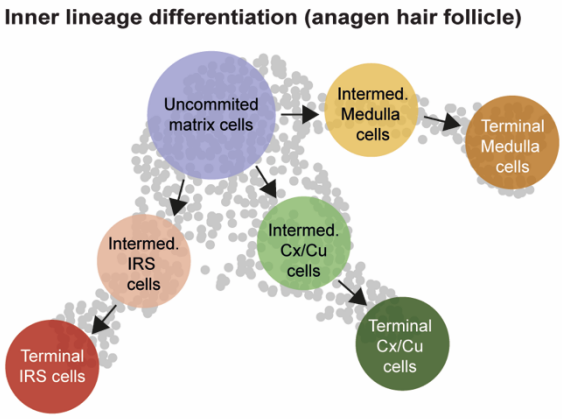

Figure 17: Scheme showcasing inner lineage differentiation from uncommitted matrix cells via intermediate stages. Overlaid on UMAP of *Msx2*$^{+}$ cells from Paper II (Figure 4). IRS: Inner root sheath. Cx: Cortex. Cu: Cuticle. Intermed.: Intermediate [adapted from (Joost, 2019)]

Lastly, we focused on skin fibroblasts. They separated into four populations and displayed a gradual topology including a significant overlap of gene signatures. We found that fibroblast populations showed spatial separation along the proximal-distal axis (from adventitia via hypodermis to the dermis). However, two populations colocalized to the dermis. Instead of being spatially segregated, they were temporally segregated. We came to the conclusion that these two populations represent cell states rather than distinct cell types, as most likely they represent the same fibroblasts that transiently alter the expression of specific genes in response to the hair cycle.

Altogether, **Paper II** resulted in the most comprehensive characterization of full-thickness anagen and telogen mouse skin to date.

4.3 PAPER III: MOLECULAR HETEROGENEITY AND DYNAMICS OF MOUSE SKIN DURING EMBRYONIC DEVELOPMENT (MORPHOGENESIS)

***New concepts and results**: Molecular and functional fibroblast heterogeneity much earlier than previously assumed. Acute loss of Wnt inhibitors marks dermal condensate formation. Embryonic skin is rich in dermal dendritic cells. The early epidermis contains a signaling-rich, highly distinct subpopulation expressing smooth muscle genes.*

This study aimed at dissecting early embryonic skin development in the mouse. Using scRNA-seq (10x Genomics Chromium system) and smFISH, we analyzed three consecutive developmental time points (E12.5, E13.5, and E14.5). This gave us temporal resolution, to see e.g., if certain cell populations only appear at certain time points, as well as spatial resolution, to see e.g., if certain cell populations migrate to their final location during the analyzed time points.

The full dataset contained more than 30 000 single-cell transcriptomes with about 26 000 transcriptomes coming from fibroblasts, while the remaining transcriptomes represented epidermal cells, immune cells, muscle cells, neural crest-derived cells, and vessel-associated

cells. Before diving into the molecular heterogeneity, we first defined anatomical landmarks in embryonic skin to facilitate the mapping of uncovered cell populations.

Dermal fibroblasts already displayed unexpected heterogeneity and based on expression profiles, RNA velocity analysis, and tissue location we could identify and characterize fibroblasts with clear fates towards functionally distinct cell types (Figure 18). We detected adipogenic fibroblasts starting from E13.5, which is the earliest report of pre-adipocytes in mouse skin. Furthermore, we observed a gradual upregulation of multiple Wnt inhibitors in the specialized hair follicle-inducing fibroblasts called dermal condensate precursors, followed by a sharp drop in Wnt inhibitor expression upon dermal condensate commitment. Strikingly this upregulation of Wnt inhibitors was paralleled by gradual Wnt activation in the same cells. Moreover, we described a novel fibroblast subpopulation that resides within developing muscles and potentially provides functional support to those developing muscles. Finally, embryonic fibroblasts also participated in complex crosstalk with various other skin cell types to support processes such as angiogenesis, neurogenesis, and recruitment of immune cells; all of which are crucial for successful skin maturation.

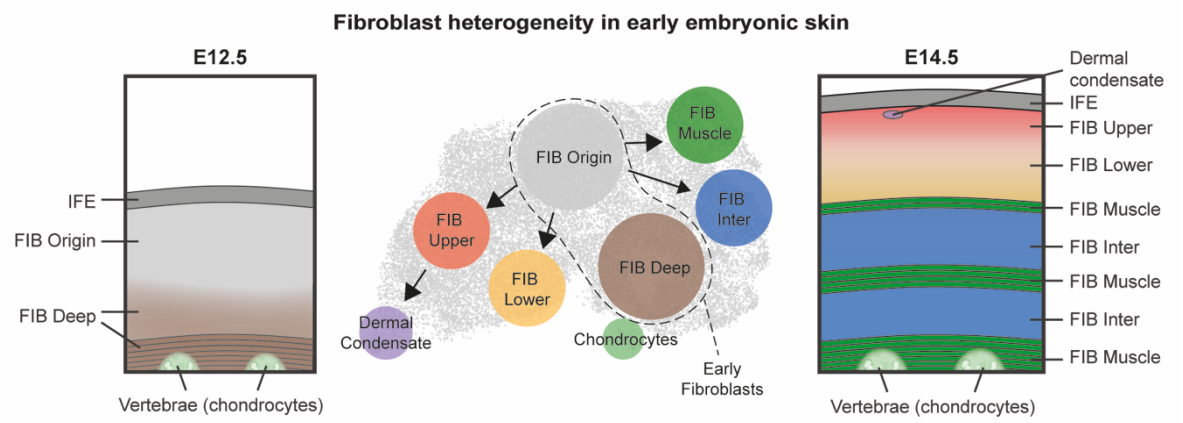

Figure 18: Left and right panel: Anatomical scheme of E12.5 and E14.5 skin, respectively. Colored by fibroblast subtypes. Central panel: Major fibroblast subtypes overlaid on UMAP of fibroblasts from Paper III (Figure 2).

We also in-depth analyzed the other stromal cell types and the most interesting findings will be highlighted hereafter. While vascular and lymphatic endothelial cells were present at all analyzed time points, we found that mural cells were only recruited at E13.5. The early cutaneous immune compartment was dominated by dermal dendritic cells, macrophages, and mast cells and it was mostly skin-resident immune cells that participated in the recruitment of more immune cells. The panniculus carnosus muscle forms around E14 and transcriptionally resembles other back muscles.

The most remarkable transformation in the skin during the analyzed embryonic period is the transformation of the single-layered, seemingly uniform epidermis to a multi-layered and appendage-producing epidermis. We were able to transcriptionally capture that transformation. We furthermore uncovered striking heterogeneity within the E12.5 epidermis with one population showing a strong resemblance of later basal IFE cells, while the other population displayed a very distinct signature and was enriched in signaling molecules and smooth muscle

genes. We robustly identified cells of the protective embryonic layer called periderm, which allowed for a detailed description of their molecular identity as well as their molecular maturation. We were able to model early embryonic epidermal stratification and our imaging data suggests that the differentiation program is initiated prior to delamination. Lastly, we found evidence that already immediately after their formation, both the hair placode as well as the dermal condensate engage in reciprocal interactions with major cell types to ensure that the newly formed hair follicle is properly innervated, surrounded by blood vessels, and supported by immune cells.

In sum, **Paper III** provides a meticulous dissection of early embryonic skin. It uncovered novel cellular heterogeneity and shed light on the complex interplay of different cell types that are required for proper skin maturation.

4.4 PAPER IV: MOLECULAR DYNAMICS OF MOUSE SKIN DURING WOUND HEALING (TISSUE REGENERATION)

New concepts and results: *A novel computational approach to identify keratinocytes contributing to wound healing. Epidermal progeny originating from distinct niches converges upon wound contribution. Wound response in epidermal cells starts prior to exit from their niche.*

This study employed a powerful combination of genetic lineage tracing and scRNA-seq to study wound response in the mouse epidermis at single-cell resolution. This approach allowed us to follow distinct epidermal cell populations during their journey to the newly formed wound epidermis and to monitor their individual transcriptional adaptations.

We used Lgr5-EGFP-IRES-CreERT2/R26-tdTomato and Lgr6-EGFP-IRES-CreERT2/R26-tdTomato mice to genetically label two molecularly and functionally distinct populations of epidermal stem cells: *Lgr5*$^{+}$ cells located in the hair follicle bulge or *Lgr6*$^{+}$ cells located in the interfollicular epidermis and hair follicle isthmus, respectively. Tracing was initiated prior to full-thickness dorsal wounding, and samples were collected 1 day, 4 days, 7 days, 10 days, and more than one month post-wounding. Additionally, non-wounded control samples were collected. FACS-sorted TOMATO^{+} cells underwent scRNA-seq using the STRT-C1 platform and the final dataset contained 1 873 cells.

It was inevitable that sorted cells would contain both, unresponsive cells still residing in their original niche and responsive cells migrating towards the wound. Thus, the first important achievement of this study was to computationally identify those cells that had truly responded to the injury for further analysis. This was done using a naïve Bayes classifier which identified expression profiles significantly differing from expression profiles observed in the homeostatic setting.

Subsequently, those wound cells were clustered. Interestingly they did not primarily cluster by sampling time point but formed distinct wound cell states, which peaked at different time points post-wounding, that persisted over several sampling time points, and that co-existed within the

same wounds. Comparing those wound cell states was thus preferable over comparing collection time points, as at one time point there might be cells that had only just left their original niche while others had already progressed further along their migratory path towards the wound site.

When it comes to the cell's identity, we could show that Lgr5 progeny transcriptionally adapts to wound healing very rapidly and before even exiting their niche. They upregulate an IFE-like signature and rapidly remodel their receptor gene expression which enables them to respond to wound-derived stromal ligands. As the Lgr5 progeny migrates towards the wound site, they gradually lose their bulge identity and become part of the IFE lineage. The majority of Lgr6 cells possessed an IFE-like signature from the very beginning and we could show that they were furthermore already primed to respond to wound signals prior to wounding.

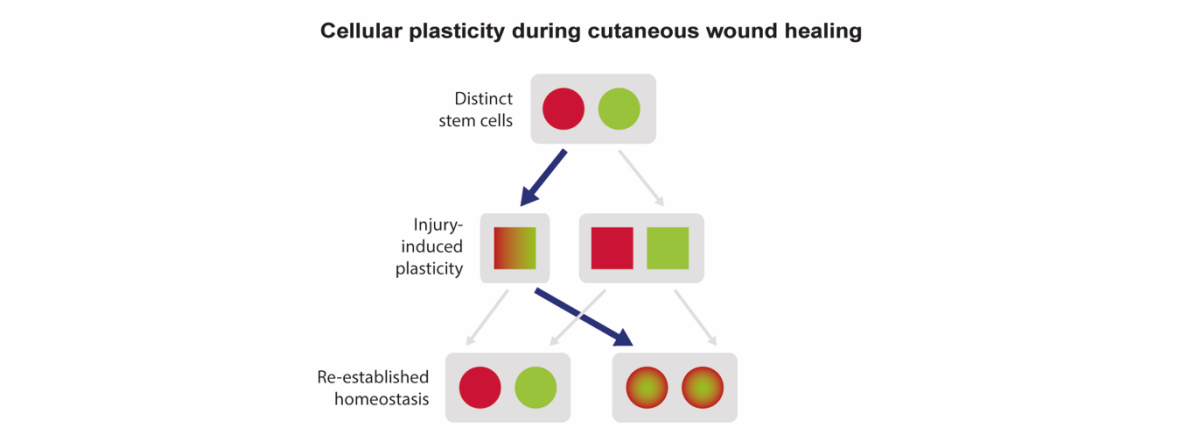

Figure 19: Scheme showcasing the different possibilities for cellular plasticity during cutaneous wound healing. Distinct starting populations are marked in red and green, respectively. Upon injury cells could converge to a common injury-induced cell state (symbolized by a square with a gradient) or they could all undergo their own injury-induced molecular changes. Upon re-establishment of homeostasis, cells could either revert back to their original state or acquire a common new identity (symbolized by a circle with a gradient).
[inspired by (Donati and Watt, 2015), and adapted from (Joost, 2019)]

This demonstrates that Lgr5 progeny gradually converges towards Lgr6 progeny by acquiring an IFE-like signature, wound-induced molecular programs, and an equalized interaction potential with the wound stroma (Figure 19). We could furthermore show that both Lgr5 and Lgr6 progeny pass through a functionally similar, sequential wound healing program. At the time of their highest transcriptional convergence, Lgr5 and Lgr6 progeny still maintain some molecular differences. However, rather than reflecting their distinct origins, these differences seem to reflect their location and their exposure to certain niche signals at the time of sampling.

In sum, **Paper IV** provides a beautiful example of how scRNA-seq cannot only be used to characterize tissue composition, but also to perform functional studies looking at dynamic processes such as wound healing.

5 CONCLUSIONS AND FURTHER DIRECTIONS

With the papers included in this thesis, we have contributed a systematic and unbiased dissection and characterization of mouse skin at the single-cell level. This marks an important advancement as the majority of previous analyses were either simplified by using *ex vivo* models or biased by using *a priori* defined markers or anatomical locations to define cell populations.

Using the wealth of generated data, we gained new insights on fundamental questions of stem cell biology, tissue homeostasis, and regenerative medicine, such as:

- What determines stemness?
- When and why does a cell commit to differentiation?
- Which vectors describe cellular heterogeneity within a tissue?
- How do healthy tissues achieve and accommodate regenerative processes?
- How is tissue maturation coordinated during embryonic development?
- What are the dynamics of injury-induced cellular plasticity?

Most importantly, we found that epidermal heterogeneity is almost exclusively determined by a cell's differentiation signature and its spatial signature (**Paper I**). Inner hair follicle lineages arise from uncommitted matrix progenitors and outer hair follicle lineages show a distinct subdivision into a basal and a suprabasal layer (**Paper II**). Embryonic fibroblasts display molecular heterogeneity and functional specialization much earlier than expected (**Paper III**). Epidermal cells that contribute to cutaneous wound healing undergo transcriptional adaptations before they even start to migrate (**Paper IV**).

Taken together, this thesis provides new insights into the dynamic and heterogenous nature of mouse skin during adult tissue homeostasis, embryonic development, and tissue regeneration after injury.

However, it is self-evident that the wealth of data generated within the frame of this thesis harbors more information than we were able to extract. It was thus very important to us, to make the data available to everybody in the field regardless of their computational skills. The searchable online tools that we provided together with Paper I and II (and in the future Paper III) are minable resources that hopefully will spur further exciting discoveries.

A detailed molecular understanding of homeostatic, healthy skin is also an important prerequisite for exploring pathological states such as skin cancer, chronic wounds, or inflammatory skin diseases.

Moreover, it is possible to build on the presented data by comparing embryonic skin to adult skin. As regenerative medicine often exploits developmental programs, a direct comparison might for example reveal, which adult dermal and epidermal populations are most similar to their embryonic counterparts and thus, potentially most receptive to hair follicle induction.

An ever-increasing number of publicly available datasets and large global atlas projects, such as *Tabula Muris* (Schaum et al., 2018) or the *Human Cell Atlas* (Regev et al., 2017), also allows for comparisons across tissues to see if uncovered principles of tissue homeostasis or regeneration represent universal principles or if they are tissue-specific.

While the papers included in this thesis draw a detailed picture of mouse skin and address a number of fundamental questions of stem cell biology, it remains to be shown if those findings hold true in human skin. Since we published the first scRNA-seq-based skin paper in 2016, a number of human scRNA-seq-based studies have been published [reviewed in (Dubois et al., 2021)]. It is compelling to compare our data to the newly published human data to evaluate the translational value of our findings.

As pointed out in the methods section, great advancements have been made in spatially resolved transcriptomics, and studying genome-wide transcriptomics *in situ* has now become a tangible goal. Having the transcriptomics data linked to the spatial information from the start would significantly facilitate biological interpretations compared to our two-step-approach where we have to separately map back the identified cell populations one by one.

We are also aware that gene expression only represents one level of regulating a cell's functional and molecular identity. Thus, it will be important for future research to integrate gene expression with other modalities, such as epigenetic modifications or protein expression. More and more modalities are becoming available at single-cell resolution and also the computational tools for performing integrated multimodal analysis are rapidly evolving [reviewed in (Ma et al., 2020)].

Summing up these considerations, it becomes clear that while still posing analytical and technical challenges, large-scale single-cell data has revolutionized the way we do biomedical research, and it allows us to address biological questions that were previously out of reach. By relying less on prior definitions, hypotheses and markers, biomedical research has become much more data-driven. This marks an important step and will very likely contribute to a more detailed understanding of complex biological systems.

6 POPULAR SCIENCE SUMMARY

Cells are the building blocks of life. Our body is made up of trillions of cells and each tissue has a special blend of different cell types that work in concert to fulfill the tissue's function. In the skin, two major cell types populate the two major layers of skin (Figure 20). So-called epithelial cells reside in the outermost layer of skin that faces the environment and that builds the actual barrier to the outside world. The underlying layer is made up of so-called fibroblasts that build the connective tissue, which functions as a scaffold on which the epithelial cells sit. However, for a fully functional tissue many more cell types need to contribute, and they are interspersed throughout the skin. There are pigment cells that provide pigment to epithelial cells so that they can produce a pigmented hair. There are nerves that allow for the sensation of touch and pain. There is a dense network of blood vessels that supply the skin with oxygen and nutrients. And additionally, there are fat cells deep down in the skin that store energy and serve as insulation and mechanical cushion.

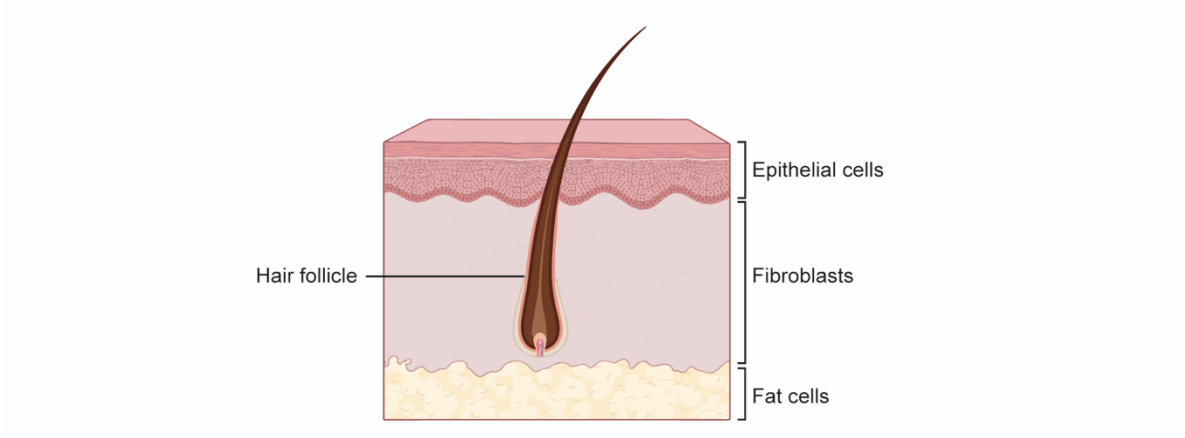

Figure 20: Architecture of skin tissue. Created with BioRender.com.

The genome holds the instructions for how to build all the different proteins that are needed for a cell to survive and fulfill its function. Each cell in our body has the same genome, yet not all the cells in our body are the same. This is because each cell only reads part of the genome and thus also produces only a subset of proteins. Depending on which proteins are produced, a cell may become an epithelial cell or a fibroblast, or any other cell type.

With a modern technology called single-cell RNA-sequencing, we are able to look at every single cell in a tissue and determine which part of the genome they are currently reading. For the parts that are read so-called mRNA is produced which in turn serves as a blueprint for protein production. Looking at the entire set of mRNAs in a cell we can conclude e.g., what type of cell we have, if the cell differs from other cells of the same type, and if this cell is in an active state or resting state. The major disadvantage of single-cell RNA-sequencing is that we don't know where in a tissue the cell, whose mRNA content and cell type identity we have uncovered using single-cell RNA-sequencing, was originally located.

This is where another method called in situ hybridization comes in. This method allows us to visualize mRNAs within tissue sections. So, if single-cell RNA-sequencing tells us that a

certain cell type is present in our tissue of interest, we can then look at some of the typical mRNAs that are only present in that cell type and see where in the intact tissue this specific cell type is located. This allows us to put all cells in a spatial context, to see if any of the cells sit in unexpected locations, and to make conclusions about their functions.

In this Ph.D. thesis, a combination of the two aforementioned methods was used to study the maintenance of healthy adult skin, the embryonic development of skin, and the processes involved in the healing of skin wounds. All of these studies were performed on mice, which is a popular model system. Mouse skin resembles human skin in many regards and thus allows us to draw valuable conclusions that often also apply to human skin.

Skin is the largest organ in mammals and serves to protect the body from external harms, such as physical damage, pathogens, radiation, fluid loss, or extreme temperatures. To fulfill all of these functions, the skin is highly specialized. Among those specializations is the outermost barrier consisting of epithelial cells that form multiple layers, with newly born cells sitting at the bottom and cells moving up as they mature before finally being shed. The epithelial barrier regularly invaginates and forms so-called 'hair follicles', which produce hair. Furthermore, there is a multitude of immune cells that patrol the skin to detect pathogens and prevent infections.

In the first part of this thesis (Paper I and II), we set out to understand the composition of the skin. We generated a detailed map of the skin including all cell types such as epithelial cells, fibroblasts, pigment cells, immune cells, and many more. When looking at the epithelial cells, we found that the typical maturation process that has been described for the epithelial barrier also holds true for cells within the hair follicles, i.e., cells facing the connective tissue are very immature, while cells facing the hair are most mature. We could furthermore show that the mRNA profile of any given epithelial cell could be roughly predicted by combining only two parameters – how mature the cell is and where it is found in the tissue (in the hair follicle, in the epithelium outside the hair follicles, or at the border). This was surprising as biology is usually much more complex. Also, we could reveal how epithelial cells work together to produce a mature hair. While it is only epithelial cells that produce the actual hair, we could observe that also the other cell types in skin changed when hair follicles started producing hair. We could see that for example pigment cells and fibroblasts change their abundance and/or molecular identity when hair is produced. This is in line with previous findings that show that epithelial cells and non-epithelial cells heavily influence each other to achieve hair production. Importantly, we made all the mRNA profiles available via an online tool so that other researchers can utilize this information in their own research.

Next, we looked at embryonic skin to understand the environment in which hair follicles and the multi-layered architecture of the epithelial barrier first arise (Paper III). We see that already very early on there are fibroblasts that are specialized for example, in supporting the formation of hair follicles, in supporting developing muscles, or in giving rise to fat cells. We find epithelial cells that have just started to mature or become a hair follicle. And we capture and describe other important developmental processes such as the formation of new blood vessels

that are needed for proper skin function. By better understanding how skin in general and hair follicles, in particular, are formed during embryonic development, we can hopefully contribute to improving skin substitutes and treatments for pathological hair loss.

Lastly, we studied wound healing (Paper IV). If an open wound is inflicted, cells from the surrounding tissue contribute to close and heal the wound. We investigated how those cells change while they move from their original location to the wound site. We saw that cells already prepare for their journey when they still sit in their original location. We furthermore saw that upon arrival at the wound site all cells – no matter what their original location or function was – become very similar and blend in. One day, these new insights will hopefully help us to achieve more efficient mobilization of cells that can help to heal non-healing wounds.

Taken together, the studies included in this Ph.D. thesis have significantly advanced our understanding of how the skin is formed during embryogenesis, how it is maintained in healthy conditions, and how it is healed upon wounding.

7 POPULÄR-WISSENSCHAFTLICHE ZUSAMMENFASSUNG

Zellen sind die Bausteine des Lebens. Unser Körper besteht aus Billionen von Zellen und jedes Gewebe weist eine spezielle Mischung verschiedener Zelltypen auf, die zusammenarbeiten um die Funktion des Gewebes zu erfüllen. In der Haut zum Beispiel gibt es zwei Hauptzelltypen, die die zwei Hauptschichten der Haut besiedeln (Abbildung 21). So genannte Epithelzellen befinden sich in der äußersten Hautschicht. Diese bildet die eigentliche Barriere zur Außenwelt. Die darunter liegende Schicht besteht aus sogenannten Fibroblasten, die das Bindegewebe bilden. Das Bindegewebe fungiert als Gerüst, auf dem die Epithelzellen sitzen. Für ein voll funktionsfähiges Gewebe müssen jedoch noch viel mehr Zelltypen beitragen und diese Zellen sind in der Haut verteilt. Es gibt Pigmentzellen, die Epithelzellen mit Pigment versorgen, sodass diese pigmentiertes Haar produzieren können. Es gibt Nerven, die das Empfinden von Berührung und Schmerz ermöglichen. Es gibt ein dichtes Netzwerk von Blutgefäßen, die die Haut mit Sauerstoff und Nährstoffen versorgen. Darüber hinaus gibt es tief in der Haut Fettzellen, die Energie speichern und als Isolierung und mechanisches Polster dienen.

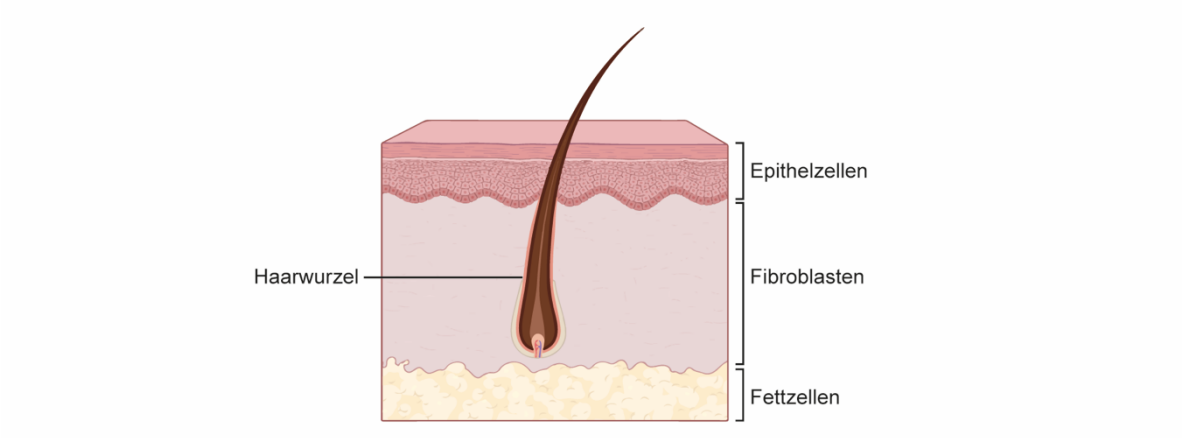

Abbildung 21: Aufbau der Haut. Generiert mit BioRender.com.

Das Erbgut enthält die Produktionsanleitung für alle verschiedenen Proteine, die eine Zelle benötigt, um zu überleben und ihre Funktion zu erfüllen. Jede Zelle in unserem Körper hat das gleiche Erbgut, aber nicht jede Zelle in unserem Körper ist gleich. Das liegt daran, dass jede Zelle nur einen Teil des Erbguts abliest und somit auch nur einen Teil der Proteine produziert. Je nachdem, welche Proteine produziert werden, erhält man eine Epithelzelle, einen Fibroblasten oder einen anderen Zelltyp.

Mit einer modernen Technologie namens Einzelzell-RNA-Sequenzierung können wir jede einzelne Zelle in einem Gewebe untersuchen und bestimmen, welchen Teil des Erbguts sie gerade abliest. Für den abgelesenen Teil werden sogenannte mRNAs produziert, die wiederum als Vorlage für die Proteinproduktion dienen. Wenn wir den gesamten Satz an mRNAs in einer Zelle betrachten, können wir beispielsweise schlussfolgern, um welchen Zelltyp es sich handelt, ob sich die Zelle von anderen Zellen des gleichen Typs unterscheidet und ob sich diese Zelle gerade teilt oder in einem Ruhezustand befindet. Der größte Nachteil der Einzelzell-RNA-Sequenzierung liegt darin, dass wir nicht wissen, wo in einem Gewebe sich die Zelle

befand, deren mRNA-Gehalt und Identität wir mithilfe der Einzelzell-RNA-Sequenzierung gerade entschlüsselt haben.

Hier kommt eine andere Methode ins Spiel, die als In-situ-Hybridisierung bezeichnet wird. Mit dieser Methode können wir mRNAs in Gewebeschnitten sichtbar machen. Wenn uns die Einzelzell-RNA-Sequenzierung sagt, dass ein bestimmter Zelltyp in unserem Gewebe vorhanden ist, können wir einige der typischen mRNAs, die nur in diesem Zelltyp vorhanden sind, einfärben und sehen, wo sich dieser spezifische Zelltyp im intakten Gewebe befindet. Dies ermöglicht es uns, alle Zellen in einen räumlichen Kontext zu bringen. So können wir sehen ob sich die Zellen möglicherweise an unerwarteten Orten befinden und wir können Schlussfolgerungen über ihre Funktionen ziehen.

In dieser Doktorarbeit wurde eine Kombination der beiden oben genannten Methoden verwendet, um zu untersuchen, wie Haut während der Embryonalentwicklung entsteht, wie gesunde Haut erhalten und erneuert wird, und welche Prozesse bei der Wundheilung ablaufen. All diese Untersuchungen wurden an Mäusen durchgeführt, welche ein beliebtes Modellsystem sind. Die Haut der Maus ähnelt in vielerlei Hinsicht der menschlichen Haut und ermöglicht es oft, wertvolle Schlussfolgerungen auch für die menschliche Haut zu ziehen.

Die Haut ist das größte Organ bei Säugetieren und dient dazu, den Körper vor Verletzungen, Krankheitserregern, Strahlung, Flüssigkeitsverlust oder extremen Temperaturen zu schützen. Um all diese Funktionen zu erfüllen, ist die Haut hochspezialisiert. Zu diesen Spezialisierungen gehört die Epithelbarriere mit einer typischen mehrschichtigen Architektur, bei der neugeborene Zellen unten sitzen und sich im Laufe ihrer Reifung nach oben bewegen, bevor sie schließlich abgestoßen werden. Die Epithelbarriere ist jedoch keine flache Schicht, sondern bildet Einstülpungen, die in das darunter liegende Bindegewebe hineinwachsen. Diese Einstülpungen bestehen aus Epithelzellen, die Haare produzieren, und werden als „Haarwurzel“ bezeichnet. Darüber hinaus gibt es eine Vielzahl von Immunzellen, die die Haut überwachen, um Krankheitserreger zu entdecken und Infektionen vorzubeugen.

Im ersten Teil dieser Arbeit (Veröffentlichung I und II) hatten wir uns zum Ziel gesetzt, die Zusammensetzung der Haut zu verstehen. Wir haben eine detaillierte Karte der Haut erstellt, die alle Zelltypen wie Epithelzellen, Fibroblasten, Pigmentzellen, Immunzellen und viele mehr enthält. Bei der Betrachtung der Epithelzellen stellten wir fest, dass der typische Reifungsprozess, der für die Epithelbarriere beschrieben ist, auch für Zellen in den Haarwurzeln gilt. Das heißt dass Zellen, die dem Bindegewebe zugewandt sind, sehr unreif sind, während Zellen, die dem produzierten Haar im Inneren der Haarwurzel zugewandt sind am ausgereiftesten sind. Wir konnten außerdem zeigen, dass das mRNA-Profil einer bestimmten Epithelzelle grob durch nur zwei Parameter vorhergesagt werden kann: Wie reif die Zelle ist und wo im Gewebe sie sitzt (in der Haarwurzel, im Epithel zwischen den Haarwurzeln oder an der Grenze). Dies war überraschend, da Biologie normalerweise viel komplexer ist. Wir konnten auch zeigen, wie Epithelzellen zusammenarbeiten um ein Haar zu produzieren. Obwohl nur Epithelzellen das eigentliche Haar produzieren, konnten wir beobachten, dass sich auch die anderen Zelltypen in der Haut verändern, wenn Haare produziert

werden. Wir konnten sehen, dass zum Beispiel Pigmentzellen und Fibroblasten ihre Häufigkeit und/oder molekulare Identität ändern, wenn Haare produziert werden. Dies steht im Einklang mit früheren Berichten die zeigen, dass sich Epithelzellen und Nicht-Epithelzellen stark gegenseitig beeinflussen um erfolgreiche Haarproduktion zu erwirken. Bemerkenswert ist auch, dass wir alle mRNA-Profile über ein Online-Tool frei verfügbar gemacht haben, damit andere Forscher diese Informationen für ihre eigenen Forschungsfragen verwenden können.

Als nächstes untersuchten wir die embryonale Haut, um die Umgebung zu verstehen, in der Haarwurzeln und die vielschichtige Architektur der Epithelbarriere zum ersten Mal entstehen (Veröffentlichung III). Wir sahen, dass es bereits sehr früh Fibroblasten gibt, die darauf spezialisiert sind, beispielsweise die Bildung von Haarwurzeln, Muskeln oder Fettzellen zu unterstützen. Wir fanden Epithelzellen, die gerade erst zu reifen begonnen haben und auch erste Epithelzellen, die eine Haarwurzel bilden werden. Gleichzeitig erfassten und beschrieben wir andere wichtige Entwicklungsprozesse wie die Bildung neuer Blutgefäße, die für eine ordnungsgemäße Hautfunktion erforderlich sind. Wenn wir besser verstehen, wie Haut im Allgemeinen und Haare im Besonderen während der Embryonalentwicklung gebildet werden, können wir hoffentlich dazu beitragen, Hautersatzprodukte und Behandlungen für pathologischen Haarausfall zu verbessern.

Zuletzt untersuchten wir die Wundheilung (Veröffentlichung IV). Um eine offene Wunde zu schließen und zu heilen, tragen Zellen aus dem umliegenden Gewebe bei. Wir haben untersucht, wie sich diese Zellen verändern, während sie sich von ihrer ursprünglichen Position zur Wunde bewegen. Wir haben gesehen, dass sich Zellen bereits auf ihre Reise vorbereiten, wenn sie sich noch an ihrem ursprünglichen Standort befinden. Wir haben außerdem beobachtet, dass sich bei der Ankunft in der Wunde alle Zellen - unabhängig von ihrem ursprünglichen Standort oder ihrer ursprünglichen Funktion - sehr ähneln und sich vermischen. Diese neuen Erkenntnisse werden es hoffentlich ermöglichen, dass wir eines Tages Zellen besser mobilisieren können, damit sie helfen nicht-heilende Wunden zu heilen.

Zusammengenommen haben die in dieser Doktorarbeit enthaltenen Studien unser Verständnis darüber, wie Haut während der Embryonalentwicklung gebildet wird, wie sie unter gesunden Bedingungen erhalten bleibt und wie sie bei Verwundung geheilt wird, erheblich erweitert.

8 ACKNOWLEDGEMENTS

"How lucky am I to have something that makes saying goodbye so hard."
Winnie the Pooh (A. A. Milne)

As my Ph.D. studies come to an end, I would like to pause and express my gratitude to all the people that inspired, taught, encouraged, accompanied, and helped me during this journey.

First and foremost, I would like to express my sincere gratitude to my supervisor **Maria Kasper**. It was your endless enthusiasm for science that made me interested in joining your lab at the very beginning. And it was your endless enthusiasm for science that managed to re-ignite my own enthusiasm when facing oh so many challenges in my research projects. Your scientific integrity – no matter what – has always impressed me, and I have very much enjoyed learning from you. I also really appreciated the balance that you found between giving me the freedom to design and run my own projects and the support and guidance you were willing to give whenever I needed it. Not to forget, that between my registration seminar and the defense of this thesis we have faced three periods of parental leave between you and me, two lab moves, and the Corona pandemic. I believe that we did very well, nevertheless. I wish you and the Kasper lab the best of luck for the future!

I would also like to thank my co-supervisors **Marco Gerling** and **Rune Toftgård** for their support and for sharing their wisdom. It was very comforting to have the two of you in the background, knowing that if anything were to happen, I could always count on you.

My sincerest thanks also go to **Lisa Westerberg** for her mentorship. I enjoyed every single one of our meetings and I am grateful for all the advice that you shared with me. Thank you so much also for agreeing to be my chairperson during the defense. It is very calming to have such a kind soul by my side.

My Ph.D. time was most significantly determined by all my great current and former colleagues in the MKA group. Karl and Simon, thanks for being my Ph.D. partners in crime! **Karl**, I really cherished all of our fun discussions which preferentially turned on microscopy, data presentation, and science podcasts. Thanks to your balanced character spiced with some quirky Estonian humor, working with you has always been a pleasure. Your feedback on the thesis was invaluable; so was your 'secret' stock of chocolate. **Simon**, while I certainly struggled at times to get you talking, I really enjoyed our fruitful collaboration and learned so much from you about statistics and scRNA-seq analysis. I also hold fond memories of the numerous joint climbing sessions with you, Karl and Maria H. **Xiaoyan**, you are such a kind and hard-working soul. It was so nice to have you around for all those years and you helped me in so many situations; while you are far too humble to ever recognize that. **Tim**, I want to thank you for all the good times spent together in the lab, or in social settings. Also, I want to thank you for your tireless efforts to educate us on immunology and the peculiarities of the English language. **Anja**, thanks for giving me such a warm welcome to the group when I first arrived. **Unni**,

thanks for daring to join the group as the first PostDoc ever and for sharing all your expertise. Dear **Sasha**, thanks for taking good care of our lab and for your invaluable help throughout the years – may it be introducing me to lab routines, helping me with stainings and mice, or taking up my hiking trousers. Thanks to all of you for being such a supportive group, that is always happy to give feedback or even help hands-on whenever a big experiment requires an extra pair of hands – or sometimes even all pairs of hands available.

I would also like to thank our temporary lab members. **Jérôme Lamartine**, we were so lucky that you spent your sabbatical in our lab. You definitely enriched our environment and taught us a lot about human skin. **Christina**, thank you so much for the fun times that we had together in the lab and for becoming such a good friend. **Agnes**, your visit to our lab as well as our cancer genomics course in Bergen will always remain cherished memories. I am very curious to see where our project on skin cancer will still lead us.

My special thanks also go to our neighboring labs. Firstly, the **RTO group** that shared oh so many lab retreats and 'fikas' with our group. While we did not really have so many opportunities to socialize in the past two years, I hold fond memories of the times we spent together. **Agneta**, thank you so much for patiently putting up with my Swedish when it was still in its baby shoes and for the absolutely best cakes. **Maryam**, thanks for all your help with the mouse surgeries and for teaching me that, in life, there is nothing that a lemon cannot fix. **Leander**, thanks for sharing all your lab advice and life experience. **Peter**, thanks for all the fresh fruit from your garden and the treats from Greece. **Maria H.** thanks for openly sharing your struggles and successes over a good cup of coffee every now and then. **Natalie**, thanks for all the nice walks and winter BBQs with Leo and Frida. **Lenny**, thanks for the fun times together and your refreshing buoyant spirit. Secondly, the **PKA group** that was brave enough to move in with us into a shared lab and office space on a new campus. I really enjoyed your lively company.

I also want to say thank you to all my other colleagues at the BioNut department for making it such a pleasant and helpful environment, and to our new colleagues at the CMB department for welcoming us with open arms. I want to thank the BioNut administration for all the help throughout the years and my special thanks go to **Monica Ahlberg** and **Lars-Arne Haldosén**. One can clearly feel that it is the Ph.D. students' best interests that guide all your decisions and actions.

I also want to thank our collaborators that contributed to my embryo project in oh so many ways; be it through discussions or practical help: **Michael Rendl** in New York. **Marja Mikkola** in Helsinki. **Beate Lichtenberger, Igor Adameyko, Adelheid Elbe-Bürger, Maria Eleni Kastriti,** and **René Reitermaier** in Vienna. **Chiara Levra Levron** and **Giacomo Donati** in Torino. **Rickard Sandberg, Christoph Ziegenhain, Paulo Czarnewski, Åsa Björklund, Maria Genander, Hong Qian, Lakshmi Sandhow** and **Thibault Bouderlique** in Stockholm. Collaborating with you was a privilege and I learned so much from all of you!

I would like to offer my special thanks to the staff at PKL, LCI, ESCG, and UPPMAX for their technical support with our projects. The **staff at the animal house (PKL)** took good care of our mice throughout the years and their help has proven especially valuable when we moved campus and they went the extra mile to help me finish the last experiments. The **staff at our imaging facility (LCI)** made sure the facility was always up to the highest standards and that support was available whenever problems arose. **Sylvie**, I could not have wished for a better teacher in microscopy!

I would also like to take this opportunity and thank all of my teachers along the way. Thanks to my **high school teachers** for sharing their passion for science. Thanks to **Jason Krajl** from the NIST institute for guiding me through my first ever wet lab experiments. Thanks to **Poh Loong Soong** and the **Zimmerman Lab** in Göttingen for sparking my interest in stem cell biology. Thanks to **Dunja Knapp** and the **Tanaka Lab** in Dresden for introducing me to axolotl research. **Elly**, you are a real role model to me. I admire you for being such an outstanding scientist and also for being a caring group leader. Thanks to **Claudia Ortlepp** and **Christian Thiede** in Dresden for giving me the chance to gain experience in the field of molecular diagnostics. Thanks to **Marco Mignardi** and **Mats Nilsson** for supervising my first steps into the field of smFISH. All of you shaped me as a scientist, and I am where I am today thanks to you.

A special thanks also goes to **KI career service** that provided me with the opportunity to do an internship with Schain Research AB during the last year of my Ph.D. and to **Frida Schain** and **Christina Jones** at Schain Research AB who made sure that this internship would be very insightful and worthwhile.

I also want to thank **Burcu, Matheus, Silke, David**, and the whole **winter conference team** for the shared good times and the joint efforts for putting together a conference worthwhile - year after year! **Sandra**, I have never met anyone as vivid and busy and as energy-radiating as you, and I really miss you.

Thanks also to my classmates **Alvaro, Lakshmi,** and **Konstantinos** from the Biomedicine master program for staying in touch for all those years. **Aline**, I really miss our sushi nights, skiing practice, and gluten-free baking challenges.

Dear **Ani**, Dear **Parisa**! What would my Ph.D. time have been without you?! You are my favorite Ph.D. buddies, and I am so happy we could share all the ups and downs that Ph.D. life brings along. I will miss our cozy get-togethers and the spontaneous pajama parties.

Sandra, thanks to our shared Thuringian upbringing, I always felt a bit closer to home when I spent time with you. I really enjoyed all the time that we spent together both during maternity leave and after.

I would also like to thank all of our other Stockholm friends, especially **Alexandra** and **Mateusz**, for accompanying us through all those years in Stockholm. When your family is far away, your friends become family and you certainly did!

Herzlicher Dank gebührt auch meinen **MolMed-Freunden aus Göttingen**. Weit verstreut haben wir alle unseren Weg gefunden und uns doch niemals aus den Augen verloren. Dank diverser Feierlichkeiten und den alljährlichen Golm-Treffen und Mädels-Wochenenden, fühle ich mich euch so nah wie eh und je.

Liebe **Nina**, Liebe **Anna**! Seit Schulzeiten seid ihr als beste Freundinnen an meiner Seite und ich mag es mir auch gar nichts anders vorstellen. Seit Studienzeiten trennen uns leider viele Kilometer, aber das hat uns zum Glück nie davon abgehalten am Leben des anderen teilzuhaben. Wir sind miteinander gewachsen. Haben gemeinsam innegehalten, wenn es mal nicht so lief. Haben gemeinsam gefeiert, wenn es einen Erfolg zu verzeichnen gab. Ich bin so froh, dass es euch gib!

Liebe **Mama**, Lieber **Papa** – Ganz lieben Dank dafür, dass ihr immer für mich da seid! Komme, was da wolle. Ihr habt es geschafft, mir gleichzeitig Wurzeln und Flügel zu verleihen und dafür bin ich euch so dankbar! Lieber **Thomas**, als vorbildlicher großer Bruder hast du dich schon früh um meine Ausbildung gesorgt. Ich bin mir nicht sicher, was sich mehr ausgezahlt hat – das gemeinsame Winterreifen aufziehen oder das gemeinsame Lösen von P.M. Logik Trainer Rätseln. Liebe **Steffi**, meine Liebe zum Reisen und die Weltoffenheit habe ich definitiv dir zu verdanken. Du warst schon immer mein großes Vorbild. Liebe **Kristin**, Liebe **Anabella**, Liebe **Sofia**, Lieber **Andreas**, Lieber **Erik**, Lieber **Hagen**, und Lieber **Felix** - Ich bin so froh, euch alle zu haben!

Liebe **Kerstin**, Lieber **Klaus**, Liebe **Anja** – Vielen Dank dafür, dass ihr mir zu einer zweiten Familie geworden seid!

Der größte Dank aber gebührt meinem lieben **Stefan**! Du bist mein zu Hause. Mein Heute und mein Morgen. Wir gehen unseren Weg gemeinsam mit unserer kleinen wundervollen **Frida** – und das ist es, was wirklich zählt. Ich danke dir für all deine Geduld, Aufmunterung und deine Unterstützung! Unsere geteilte Liebe zur Wissenschaft hat dich glücklicherweise für so manchen unmöglichen experimentellen Zeitplan Verständnis haben lassen.

9 REFERENCES

Abdo, H., Calvo-Enrique, L., Lopez, J.M., Song, J., Zhang, M.D., Usoskin, D., Manira, A. El, Adameyko, I., Hjerling-Leffler, J., and Ernfors, P. (2019). Specialized cutaneous schwann cells initiate pain sensation. Science *365*, 695–699.

Aberer, W., Romani, N., Elbe, A., and Stingl, G. (1986). Effects of physicochemical agents on murine epidermal Langerhans cells and Thy-1-positive dendritic epidermal cells. J. Immunol. *136*, 1210 LP – 1216.

Achim, K., Pettit, J.B., Saraiva, L.R., Gavriouchkina, D., Larsson, T., Arendt, D., and Marioni, J.C. (2015). High-throughput spatial mapping of single-cell RNA-seq data to tissue of origin. Nat. Biotechnol. *33*, 503–509.

Adachi, T., Kobayashi, T., Sugihara, E., Yamada, T., Ikuta, K., Pittaluga, S., Saya, H., Amagai, M., and Nagao, K. (2015). Hair follicle–derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. Nat. Med. *21*, 1272–1279.

Adameyko, I., Lallemend, F., Aquino, J.B., Pereira, J. a., Topilko, P., Müller, T., Fritz, N., Beljajeva, A., Mochii, M., Liste, I., et al. (2009). Schwann Cell Precursors from Nerve Innervation Are a Cellular Origin of Melanocytes in Skin. Cell *139*, 366–379.

Ahtiainen, L., Lefebvre, S., Lindfors, P.H., Renvoisé, E., Shirokova, V., Vartiainen, M.K., Thesleff, I., and Mikkola, M.L. (2014). Directional Cell Migration, but Not Proliferation, Drives Hair Placode Morphogenesis. Dev. Cell *28*, 588–602.

Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.A., Lai, K., Ahn, R., Corbin, K., Lowe, M.M., Scharschmidt, T.C., et al. (2017). Regulatory T Cells in Skin Facilitate Epithelial Stem Cell Differentiation. Cell *169*, 1119–1129.

Alonso, L., and Fuchs, E. (2006). The hair cycle. J. Cell Sci. *119*, 391–393.

Altman, J., and Das, G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol. *124*, 319–335.

Andrews, T.S., and Hemberg, M. (2018). Identifying cell populations with scRNASeq. Mol. Aspects Med. *59*, 114–122.

Andrews, T.S., and Hemberg, M. (2019). M3Drop: Dropout-based feature selection for scRNASeq. Bioinformatics *35*, 2865–2867.

Ansell, D.M., Kloepper, J.E., Thomason, H.A., Paus, R., and Hardman, M.J. (2011). Exploring the “Hair Growth–Wound Healing Connection”: Anagen Phase Promotes Wound Re-Epithelialization. J. Invest. Dermatol. *131*, 518–528.

Aragona, M., Dekoninck, S., Rulands, S., Lenglez, S., Mascré, G., Simons, B.D., and Blanpain, C. (2017). Defining stem cell dynamics and migration during wound healing in mouse skin epidermis. Nat. Commun. *8*, 1–14.

Aragona, M., Sifrim, A., Malfait, M., Song, Y., Van Herck, J., Dekoninck, S., Gargouri, S., Lapouge, G., Swedlund, B., Dubois, C., et al. (2020). Mechanisms of stretch-mediated skin expansion at single-cell resolution. Nature *584*, 268–273.

Armulik, A., Genové, G., and Betsholtz, C. (2011). Pericytes: Developmental, Physiological, and Pathological Perspectives, Problems, and Promises. Dev. Cell *21*, 193–215.

Arwert, E.N., Hoste, E., and Watt, F.M. (2012). Epithelial stem cells, wound healing and cancer. Nat. Rev. Cancer *12*, 170–180.

Asarnow, D.M., Kuziel, W.A., Bonyhad, M., Tigelaar, R.E., Tucker, P.W., and Allison, J.P. (1988). Limited diversity of γδ antigen receptor genes of thy-1+ dendritic epidermal cells. Cell *55*, 837–847.

Barbieri, J.S., Wanat, K., and Seykora, J. (2014). Skin : Basic Structure and Function. In Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms, L. McManus, ed. (Elsevier Science & Technology), pp. 1134–1144.

Barker, N., Van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., et al. (2007). Identification of stem cells in small intestine and colon by marker gene

Lgr5. Nature *449*, 1003–1007.

Barresi, M.J.F., and Gilbert, S.F. (2020). Developmental Biology (Oxford University Press).

Becker, A.J., Mcculloch, E.A., and Till, J.E. (1963). Spleen Colonies Derived From Transplanted Mouse Marrow Cells. Nature *197*, 452–454.

Bendall, S.C., Davis, K.L., Amir, E.A.D., Tadmor, M.D., Simonds, E.F., Chen, T.J., Shenfeld, D.K., Nolan, G.P., and Pe'Er, D. (2014). Single-cell trajectory detection uncovers progression and regulatory coordination in human b cell development. Cell *157*, 714–725.

Bentley, D.R., Balasubramanian, S., Swerdlow, H.P., Smith, G.P., Milton, J., Brown, C.G., Hall, K.P., Evers, D.J., Barnes, C.L., Bignell, H.R., et al. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. Nature *456*, 53–59.

Bergen, V., Lange, M., Peidli, S., Wolf, F.A., and Theis, F.J. (2020). Generalizing RNA velocity to transient cell states through dynamical modeling. Nat. Biotechnol. *38*, 1408–1414.

Bertrand, J.Y., Giroux, S., Golub, R., Klaine, M., Jalil, A., Boucontet, L., Godin, I., and Cumano, A. (2005). Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proc. Natl. Acad. Sci. USA *102*, 134–139.

Betterman, K.L., and Harvey, N.L. (2018). Histological and Morphological Characterization of Developing Dermal Lymphatic Vessels. In Lymphangiogenesis - Methods and Protocols, G. Oliver, and M.L. Kahn, eds. (Springer Science+Business Media), pp. 19–36.

Bhaduri, A., Nowakowski, T.J., Pollen, A.A., and Kriegstein, A.R. (2018). Identification of cell types in a mouse brain single-cell atlas using low sampling coverage. BMC Biol. *16*, 1–10.

Bhatia, A., Singh, B., Amarji, B., and Katare, O.P. (2010). Tamoxifen-loaded liposomal topical formulation arrests hair growth in mice. Br. J. Dermatol. *163*, 412–415.

Biggs, L.C., and Mikkola, M.L. (2014). Early inductive events in ectodermal appendage morphogenesis. Semin. Cell Dev. Biol. *25–26*, 11–21.

Biggs, L.C., Mäkelä, O.J.M., Myllymäki, S.M., Das Roy, R., Närhi, K., Pispa, J., Mustonen, T., and Mikkola, M.L. (2018). Hair follicle dermal condensation forms via FGF20 primed cell cycle exit, cell motility, and aggregation. Elife *7*, 1–33.

Bjerknes, M., and Cheng, H. (1999). Clonal analysis of mouse intestinal epithelial progenitors. Gastroenterology *116*, 7–14.

Böiers, C., Carrelha, J., Lutteropp, M., Luc, S., Green, J.C.A., Azzoni, E., Woll, P.S., Mead, A.J., Hultquist, A., Swiers, G., et al. (2013). Lymphomyeloid contribution of an immune-restricted progenitor emerging prior to definitive hematopoietic stem cells. Cell Stem Cell *13*, 535–548.

Braun-Falco, O., Plewig, G., Wolff, H.H., and Burgdorf, W. (2000). Dermatology (Springer Science+Business Media).

Braun, K.M., Niemann, C., Jensen, U.B., Sundberg, J.P., Silva-Vargas, V., and Watt, F.M. (2003). Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development *130*, 5241–5255.

Brownell, I., Guevara, E., Bai, C.B., Loomis, C.A., and Joyner, A.L. (2011). Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells. Cell Stem Cell *8*, 552–565.

Butler, A., Hoffman, P., Smibert, P., Papalexi, E., and Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat. Biotechnol. *36*, 411–420.

Cabello-Aguilar, S., Alame, M., Kon-Sun-Tack, F., Fau, C., Lacroix, M., and Colinge, J. (2020). SingleCellSignalR: inference of intercellular networks from single-cell transcriptomics. Nucleic Acids Res. *48*, e55.

Calvo, F., Ege, N., Grande-Garcia, A., Hooper, S., Jenkins, R.P., Chaudhry, S.I., Harrington, K., Williamson, P., Moeendarbary, E., Charras, G., et al. (2013). Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. Nat. Cell Biol. *15*, 637–646.

Cao, R., Bråkenhielm, E., Li, X., Pietras, K., Widenfalk, J., Östman, A., Eriksson, U., and Cao, Y. (2002). Angiogenesis stimulated by PDGF-CC, a novel member in the PDGF family, involves activation of PDGFR-aa

and -ap receptors. FASEB J. *16*, 1575–1583.

Castellana, D., Paus, R., and Perez-Moreno, M. (2014). Macrophages Contribute to the Cyclic Activation of Adult Hair Follicle Stem Cells. PLoS Biol. *12*.

Cetera, M., Leybova, L., Woo, F.W., Deans, M., and Devenport, D. (2017). Planar cell polarity-dependent and independent functions in the emergence of tissue-scale hair follicle patterns. Dev. Biol. *428*, 188–203.

Chen, F., Wassie, A.T., Cote, A.J., Sinha, A., Alon, S., Asano, S., Daugharthy, E.R., Chang, J.B., Marblestone, A., Church, G.M., et al. (2016). Nanoscale imaging of RNA with expansion microscopy. Nat. Methods *13*, 679–684.

Chen, J., Suo, S., Tam, P.P., Han, J.D.J., Peng, G., and Jing, N. (2017). Spatial transcriptomic analysis of cryosectioned tissue samples with Geo-seq. Nat. Protoc. *12*, 566–580.

Chen, K.H., Boettiger, A.N., Moffitt, J.R., Wang, S., and Zhuang, X. (2015). Spatially resolved, highly multiplexed RNA profiling in single cells. Science *348*, 1360–1363.

Chen, T., Heller, E., Beronja, S., Oshimori, N., Stokes, N., and Fuchs, E. (2012). An RNA interference screen uncovers a new molecule in stem cell self-renewal and long-term regeneration. Nature *485*, 104–108.

Chen, X., Teichmann, S.A., and Meyer, K.B. (2018a). From Tissues to Cell Types and Back: Single-Cell Gene Expression Analysis of Tissue Architecture. Annu. Rev. Biomed. Data Sci. *1*, 29–51.

Chen, X., Sun, Y.C., Church, G.M., Lee, J.H., and Zador, A.M. (2018b). Efficient in situ barcode sequencing using padlock probe-based BaristaSeq. Nucleic Acids Res. *46*, 1–10.

Cheng, C.C., Tsutsui, K., Taguchi, T., Sanzen, N., Nakagawa, A., Kakiguchi, K., Yonemura, S., Tanegashima, C., Keeley, S.D., Kiyonari, H., et al. (2018). Hair Follicle Epidermal Stem Cells Define a Niche for Tactile Sensation. Elife *7:e38883*, 1–29.

Choi, H.M.T., Chang, J.Y., Trinh, L.A., Padilla, J.E., Fraser, S.E., and Pierce, N.A. (2010). Programmable in situ amplification for multiplexed imaging of mRNA expression. Nat. Biotechnol. *28*, 1208–1212.

Chorro, L., Sarde, A., Li, M., Woollard, K.J., Chambon, P., Malissen, B., Kissenpfennig, A., Barbaroux, J.B., Groves, R., and Geissmann, F. (2009). Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. J. Exp. Med. *206*, 3089–3100.

Clavel, C., Grisanti, L., Zemla, R., Rezza, A., Barros, R., Sennett, R., Mazloom, A.R., Chung, C.Y., Cai, X., Cai, C.L., et al. (2012). Sox2 in the Dermal Papilla Niche Controls Hair Growth by Fine-Tuning BMP Signaling in Differentiating Hair Shaft Progenitors. Dev. Cell *23*, 981–994.

Clayton, E., Doupé, D.P., Klein, A.M., Winton, D.J., Simons, B.D., and Jones, P.H. (2007). A single type of progenitor cell maintains normal epidermis. Nature *446*, 185–189.

Clevers, H. (2015). What is an adult stem cell. Science *350*.

Cockburn, K., Annusver, K., Ganesan, S., Mesa, K.R., Kawaguchi, K., Kasper, M., and Greco, V. (2021). Gradual differentiation uncoupled from cell cycle exit generates heterogeneity in the epidermal stem cell layer. BioRxiv.

Constantinou, J., Reynolds, M.L., Woolf, C.J., Safieh-Garabedian, B., and Fitzgerald, M. (1994). Nerve growth factor levels in developing rat skin: upregulation following skin wounding. Neuroreport *5*, 2281–2284.

Correa-Gallegos, D., Jiang, D., Christ, S., Ramesh, P., Ye, H., Wannemacher, J., Kalgudde Gopal, S., Yu, Q., Aichler, M., Walch, A., et al. (2019). Patch repair of deep wounds by mobilized fascia. Nature *576*, 287–292.

Cotsarelis, G., Sun, T.T., and Lavker, R.M. (1990). Label-retaining cells reside in the bulge area of pilosebaceous unit: Implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell *61*, 1329–1337.

Cunha, G.R., and Hom, Y.K. (1996). Role of mesenchymal-epithelial interactions in mammary gland development. J. Mammary Gland Biol. Neoplasia *1*, 21–35.

Dahmann, C., Oates, A.C., and Brand, M. (2011). Boundary formation and maintenance in tissue development. Nat. Rev. Genet. *12*, 43–55.

Dalessandri, T., Crawford, G., Hayes, M., Castro Seoane, R., and Strid, J. (2016). IL-13 from intraepithelial

lymphocytes regulates tissue homeostasis and protects against carcinogenesis in the skin. Nat. Commun. *7*, 1–12.

Damen, M., Soroka, E., Khatif, H., Kukat, C., Simons, B.D., and Bazzi, H. (2020). Epidermal stratification is uncoupled from centrosome-dependent cell division orientation of the basal progenitors. BioRxiv.

David, L., Feige, J.J., and Bailly, S. (2009). Emerging role of bone morphogenetic proteins in angiogenesis. Cytokine Growth Factor Rev. *20*, 203–212.

Dekoninck, S., Hannezo, E., Sifrim, A., Miroshnikova, Y.A., Aragona, M., Malfait, M., Gargouri, S., de Neunheuser, C., Dubois, C., Voet, T., et al. (2020). Defining the Design Principles of Skin Epidermis Postnatal Growth. Cell *181*, 604-620.e22.

Detmar, M. (2000). The role of VEGF and thrombospondins in skin angiogenesis. J. Dermatol. Sci. *24*, 78–84.

Dhouailly, D., and Oftedal, O.T. (2016). Integument and Associated Integumentary Appendages (Elsevier Inc.).

van Dijk, D., Sharma, R., Nainys, J., Yim, K., Kathail, P., Carr, A.J., Burdziak, C., Moon, K.R., Chaffer, C.L., Pattabiraman, D., et al. (2018). Recovering Gene Interactions from Single-Cell Data Using Data Diffusion. Cell *174*, 716-729.e27.

Donati, G., and Watt, F.M. (2015). Stem cell heterogeneity and plasticity in epithelia. Cell Stem Cell *16*, 465–476.

Donati, G., Proserpio, V., Lichtenberger, B.M., Natsuga, K., Sinclair, R., Fujiwara, H., and Watt, F.M. (2014). Epidermal Wnt/β-catenin signaling regulates adipocyte differentiation via secretion of adipogenic factors. Proc. Natl. Acad. Sci. USA *111*, E1501-9.

Donati, G., Rognoni, E., Hiratsuka, T., Liakath-Ali, K., Hoste, E., Kar, G., Kayikci, M., Russell, R., Kretzschmar, K., Mulder, K.W., et al. (2017). Wounding induces dedifferentiation of epidermal Gata6 + cells and acquisition of stem cell properties. Nat. Cell Biol. *19*, 603–613.

Le Douarin, N.M., Creuzet, S., Couly, G., and Dupin, E. (2004). Neural crest cell plasticity and its limits. Development *131*, 4637–4650.

Doucet, Y.S., Woo, S.H., Ruiz, M.E., and Owens, D.M. (2013). The Touch Dome Defines an Epidermal Niche Specialized for Mechanosensory Signaling. Cell Rep. *3*, 1759–1765.

Doupé, D.P., Klein, A.M., Simons, B.D., and Jones, P.H. (2010). The Ordered Architecture of Murine Ear Epidermis Is Maintained by Progenitor Cells with Random Fate. Dev. Cell *18*, 317–323.

Doupé, D.P., Alcolea, M.P., Roshan, A., Zhang, G., Klein, A.M., Simons, B.D., and Jones, P.H. (2012). A single progenitor population switches behavior to maintain and repair esophageal epithelium. Science *337*, 1091–1093.

Driskell, R.R., and Watt, F.M. (2015). Understanding fibroblast heterogeneity in the skin. Trends Cell Biol. *25*, 92–99.

Driskell, R.R., Giangreco, A., Jensen, K.B., Mulder, K.W., and Watt, F.M. (2009). Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. Development *136*, 2815–2823.

Driskell, R.R., Lichtenberger, B.M., Hoste, E., Kretzschmar, K., Simons, B.D., Charalambous, M., Ferron, S.R., Herault, Y., Pavlovic, G., Ferguson-Smith, A.C., et al. (2013). Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature *504*, 277–281.

Driskell, R.R., Jahoda, C.A.B.B., Chuong, C.-M.M., Watt, F.M., and Horsley, V. (2014). Defining dermal adipose tissue. Exp. Dermatol. *23*, 629–631.

Dubois, A., Gopee, N., Olabi, B., and Haniffa, M. (2021). Defining the Skin Cellular Community Using Single-Cell Genomics to Advance Precision Medicine. J. Invest. Dermatol. *141*, 255–264.

Dulauroy, S., Di Carlo, S.E., Langa, F., Eberl, G., and Peduto, L. (2012). Lineage tracing and genetic ablation of ADAM12 + perivascular cells identify a major source of profibrotic cells during acute tissue injury. Nat. Med. *18*, 1262–1270.

Efremova, M., Vento-Tormo, M., Teichmann, S.A., and Vento-Tormo, R. (2020). CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. Nat. Protoc. *15*, 1484–1506.

Elbe, A., Tschachler, E., Steiner, G., Binder, A., Wolff, K., and Stingl, G. (1989). Maturational steps of bone marrow-derived dendritic murine epidermal cells. Phenotypic and functional studies on Langerhans cells and

Thy-1+ dendritic epidermal cells in the perinatal period. J. Immunol. *143*, 2431–2438.

Ellis, S.J., Gomez, N.C., Levorse, J., Mertz, A.F., Ge, Y., and Fuchs, E. (2019). Distinct modes of cell competition shape mammalian tissue morphogenesis. Nature *569*, 497–502.

Eraslan, G., Simon, L.M., Mircea, M., Mueller, N.S., and Theis, F.J. (2019). Single-cell RNA-seq denoising using a deep count autoencoder. Nat. Commun. *10*, 1–14.

Erickson, C.A. (1993). From the Crest to the Periphery: Control of Pigment Cell Migration and Lineage Segregation. Pigment Cell Res. *6*, 336–347.

De Falco, M., Pisano, M.M., and De Luca, A. (2014). Embryology and Anatomy of the Skin. In Current Clinical Pathology. Skin Cancer., A. Baldi, ed. (New York: Springer Science+Business Media), pp. 1–15.

Feil, R., Wagner, J., Metzger, D., and Chambon, P. (1997). Regulation of Cre Recombinase Activity by Mutated Receptor Ligand-Binding Domains. Biochem. Biophys. Res. Commun. *237*, 753–757.

Festa, E., Fretz, J., Berry, R., Schmidt, B., Rodeheffer, M., Horowitz, M., and Horsley, V. (2011). Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. Cell *146*, 761–771.

Finnegan, A., Cho, R.J., Luu, A., Harirchian, P., Lee, J., Cheng, J.B., and Song, J.S. (2019). Single-cell transcriptomics reveals spatial and temporal turnover of keratinocyte differentiation regulators. Front. Genet. *10*, 1–14.

Fogg, D., Sibon, C., Chaouki, M., Jung, S., Aucouturier, P., Littman, D.R., Cumano, A., and Geissmann, F. (2006). A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells. Science *311*, 83–88.

Frank, E., and Sanes, J.R. (1991). Lineage of neurons and glia in chick dorsal root ganglia: Analysis in vivo with a recombinant retrovirus. Development *111*, 895–908.

Fuchs, E. (1990). Epidermal differentiation: The bare essentials. J. Cell Biol. *111*, 2807–2814.

Fuchs, E. (2007). Scratching the surface of skin development. Nature *445*, 834–842.

Fujiwara, H., Ferreira, M., Donati, G., Marciano, D.K., Linton, J.M., Sato, Y., Hartner, A., Sekiguchi, K., Reichardt, L.F., and Watt, F.M. (2011). The basement membrane of hair follicle stem cells is a muscle cell niche. Cell *144*, 577–589.

Füllgrabe, A., Joost, S., Are, A., Jacob, T., Sivan, U., Haegebarth, A., Linnarsson, S., Simons, B.D., Clevers, H., Toftgård, R., et al. (2015). Dynamics of Lgr6+ progenitor cells in the hair follicle, sebaceous gland, and interfollicular epidermis. Stem Cell Reports *5*, 843–855.

Furlan, A., La Manno, G., Lübke, M., Häring, M., Abdo, H., Hochgerner, H., Kupari, J., Usoskin, D., Airaksinen, M.S., Oliver, G., et al. (2016). Visceral motor neuron diversity delineates a cellular basis for nipple- and pilo-erection muscle control. Nat. Neurosci. *19*, 1331–1340.

Gall, J.G., and Pardue, M.L. (1969). Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. USA *63*, 378–383.

Gay, D., Kwon, O., Zhang, Z., Spata, M., Plikus, M. V, Holler, P.D., Ito, M., Yang, Z., Treffeisen, E., Kim, C.D., et al. (2013). Fgf9 from dermal γδ T cells induces hair follicle neogenesis after wounding. Nat. Med. *19*, 916–923.

Ge, Y., Gomez, N.C., Adam, R.C., Yuan, S., Elemento, O., Fuchs, E., Ge, Y., Gomez, N.C., Adam, R.C., Nikolova, M., et al. (2017). Stem Cell Lineage Infidelity Drives Wound Repair and Article Stem Cell Lineage Infidelity Drives Wound Repair and Cancer. Cell *169*, 636–642.

Genander, M., Cook, P.J., Ramsköld, D., Keyes, B.E., Mertz, A.F., Sandberg, R., and Fuchs, E. (2014). BMP Signaling and Its pSMAD1/5 Target Genes Differentially Regulate Hair Follicle Stem Cell Lineages. Cell Stem Cell *15*, 619–633.

Gentek, R., Ghigo, C., Hoeffel, G., Bulle, M.J., Msallam, R., Gautier, G., Launay, P., Chen, J., Ginhoux, F., and Bajénoff, M. (2018a). Hemogenic Endothelial Fate Mapping Reveals Dual Developmental Origin of Mast Cells. Immunity *48*, 1160-1171.e5.

Gentek, R., Ghigo, C., Hoeffel, G., Jorquera, A., Msallam, R., Wienert, S., Klauschen, F., Ginhoux, F., and Bajénoff, M. (2018b). Epidermal γδ T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. J. Exp. Med. *215*, 2994–3005.

Gerhardt, H., and Betsholtz, C. (2003). Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res. *314*, 15–23.

Gerhardt, H., Golding, M., Fruttiger, M., Ruhrberg, C., Lundkvist, A., Abramsson, A., Jeltsch, M., Mitchell, C., Alitalo, K., Shima, D., et al. (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J. Cell Biol. *161*, 1163–1177.

Geyfman, M., Plikus, M. V., Treffeisen, E., Andersen, B., and Paus, R. (2015). Resting no more: Re-defining telogen, the maintenance stage of the hair growth cycle. Biol. Rev. *90*, 1179–1196.

Gierahn, T.M., Wadsworth II, M.H., Hughes, T.K., Bryson, B.D., Butler, A., Satija, R., Fortune, S., Love, J.C., and Shalek, A.K. (2017). Seq-Well: A Portable, Low-Cost Platform for High-Throughput Single-Cell RNA-Seq of Low-Input Samples. Nat Methods *14*, 395–398.

Ginhoux, F., and Merad, M. (2010). Ontogeny and homeostasis of Langerhans cells. Immunol. Cell Biol. *88*, 387–392.

Girardi, M., Lewis, J., Glusac, E., Filler, R.B., Geng, L., Hayday, A.C., and Tigelaar, R.E. (2002). Resident skin-specific γδ T cells provide local, nonredundant regulation of cutaneous inflammation. J. Exp. Med. *195*, 855–867.

Glover, J.D., Wells, K.L., Matthäus, F., Painter, K.J., Ho, W., Riddell, J., Johansson, J.A., Ford, M.J., Jahoda, C.A.B., Klika, V., et al. (2017). Hierarchical patterning modes orchestrate hair follicle morphogenesis. PLoS Biol. *15*, 1–31.

Gomez, C., Chua, W., Miremadi, A., Quist, S., Headon, D.J., and Watt, F.M. (2013). The interfollicular epidermis of adult mouse tail comprises two distinct cell lineages that are differentially regulated by Wnt, Edaradd, and Lrig1. Stem Cell Reports *1*, 19–27.

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., De Bruijn, M.F., Geissmann, F., et al. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature *518*, 547–551.

Goumans, M.-J., and Mummery, C. (2000). Function analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. Int. J. Dev. Biol. *44*, 253–265.

Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H.A., Stokes, N., dela Cruz-Racelis, J., and Fuchs, E. (2009). A Two-Step Mechanism for Stem Cell Activation during Hair Regeneration. Cell Stem Cell *4*, 155–169.

Grisanti, L., Clavel, C., Cai, X., Rezza, A., Tsai, S.-Y., Sennett, R., Mumau, M., Cai, C.-L., and Rendl, M. (2012). Tbx18 Targets Dermal Condensates for Labeling, Isolation, and Gene Ablation during Embryonic Hair Follicle Formation. J. Invest. Dermatol. *133*, 344–353.

Grundberg, I., Kiflemariam, S., Mignardi, M., Imgenberg-Kreuz, J., Edlund, K., Micke, P., Sundström, M., Sjöblom, T., Botling, J., and Nilsson, M. (2013). In situ mutation detection and visualization of intratumor heterogeneity for cancer research and diagnostics. Oncotarget *4*, 2407–2418.

Gudjonsson, J.E., Johnston, A., Dyson, M., Valdimarsson, H., and Elder, J.T. (2007). Mouse models of psoriasis. J. Invest. Dermatol. *127*, 1292–1308.

Gupta, K., Levinsohn, J., Linderman, G., Chen, D., Sun, T.Y., Dong, D., Taketo, M.M., Bosenberg, M., Kluger, Y., Choate, K., et al. (2019). Single-Cell Analysis Reveals a Hair Follicle Dermal Niche Molecular Differentiation Trajectory that Begins Prior to Morphogenesis. Dev. Cell *48*, 17-31.e6.

Gur-Cohen, S., Yang, H., Baksh, S.C., Miao, Y., Levorse, J., Kataru, R.P., Liu, X., de la Cruz-Racelis, J., Mehrara, B., and Fuchs, E. (2019). Stem cell – driven lymphatic remodeling coordinates tissue regeneration. Science *366*, 1218–1225.

Hafemeister, C., and Satija, R. (2019). Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression. Genome Biol. *20*, 1–15.

Hagemann-Jensen, M., Ziegenhain, C., Chen, P., Ramsköld, D., Hendriks, G.J., Larsson, A.J.M., Faridani, O.R., and Sandberg, R. (2020). Single-cell RNA counting at allele and isoform resolution using Smart-seq3. Nat. Biotechnol. *38*, 708–714.

Haghverdi, L., Buettner, F., and Theis, F.J. (2015). Diffusion maps for high-dimensional single-cell analysis of differentiation data. Bioinformatics *31*, 2989–2998.

Haghverdi, L., Büttner, M., Wolf, F.A., Buettner, F., and Theis, F.J. (2016). Diffusion pseudotime robustly

reconstructs lineage branching. Nat. Methods *13*, 845–848.

Haghverdi, L., Lun, A.T.L., Morgan, M.D., and Marioni, J.C. (2018). Correcting batch effects in single-cell RNA sequencing data by matching mutual nearest neighbours. Nat. Biotechnol. *36*, 421–427.

Halpern, K.B., Shenhav, R., Matcovitch-Natan, O., Tóth, B., Lemze, D., Golan, M., Massasa, E.E., Baydatch, S., Landen, S., Moor, A.E., et al. (2017). Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. Nature *542*, 1–5.

Hardy, M.H. (1992). The secret life of the hair follicle. Trends Genet. *8*, 55–61.

Harper, R.A., and Grove, G. (1979). Human skin fibroblasts derived from papillary and reticular dermis: Differences in growth potential in vitro. Science *204*, 526–527.

Harshuk-Shabso, S., Dressler, H., Niehrs, C., Aamar, E., and Enshell-Seijffers, D. (2020). Fgf and Wnt signaling interaction in the mesenchymal niche regulates the murine hair cycle clock. Nat. Commun. *11*, 1–14.

Havran, W.L., and Allison, J.P. (1988). Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. Nature *335*, 443–445.

Hayashi, C., Sonoda, T., Nakano, T., Nakayama, H., and Kitamura, Y. (1985). Mast-cell precursors in the skin of mouse embryos and their deficiency in embryos of Sl/Sld genotype. Dev. Biol. *109*, 234–241.

Hayday, A.C. (2009). γδ T Cells and the Lymphoid Stress-Surveillance Response. Immunity *31*, 184–196.

Heitman, N., Sennett, R., Mok, K.W., Saxena, N., Srivastava, D., Martino, P., Grisanti, L., Wang, Z., Ma'ayan, A., Rompolas, P., et al. (2020). Dermal sheath contraction powers stem cell niche relocation during hair cycle regression. Science *367*, 161–166.

Hie, B., Bryson, B., and Berger, B. (2019). Efficient integration of heterogeneous single-cell transcriptomes using Scanorama. Nat. Biotechnol. *37*, 685–691.

Hirobe, T., and Enami, H. (2019). Histochemical study of the distribution of epidermal melanoblasts and melanocytes in Asian human skin. Ski. Res. Technol. *25*, 299–304.

Ho, A.W., and Kupper, T.S. (2019). T cells and the skin: from protective immunity to inflammatory skin disorders. Nat. Rev. Immunol. *19*, 490–502.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., Leboeuf, M., Low, D., Oller, G., Almeida, F., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. J. Exp. Med. *209*, 1167–1181.

Hoeffel, G., Chen, J., Lavin, Y., Low, D., Almeida, F.F., See, P., Beaudin, A.E., Lum, J., Low, I., Forsberg, E.C., et al. (2015). C-Myb+ Erythro-Myeloid Progenitor-Derived Fetal Monocytes Give Rise to Adult Tissue-Resident Macrophages. Immunity *42*, 665–678.

Holstege, G., and Blok, B.F. (1989). Descending pathways to the cutaneus trunci muscle motoneuronal cell group in the cat. J. Neurophysiol. *62*, 1260–1269.

Horsley, V., Aliprantis, A.O., Polak, L., Glimcher, L.H., and Fuchs, E. (2008). NFATc1 Balances Quiescence and Proliferation of Skin Stem Cells. Cell *132*, 299–310.

Hsu, Y.-C., Li, L., and Fuchs, E. (2014a). Emerging interactions between skin stem cells and their niches. Nat. Med. *20*, 847–856.

Hsu, Y.-C., Li, L., and Fuchs, E. (2014b). Transit-amplifying cells orchestrate stem cell activity and tissue regeneration. Cell *157*, 935–949.

Hsu, Y.C., Pasolli, H.A., and Fuchs, E. (2011). Dynamics between stem cells, niche, and progeny in the hair follicle. Cell *144*, 92–105.

Iannone, M., Janowska, A., Dini, V., Tonini, G., Oranges, T., and Romanelli, M. (2019). Itch in Chronic Wounds: Pathophysiology, Impact, and Management. Medicines *6*.

Ipponjima, S., Hibi, T., and Nemoto, T. (2016). Three-dimensional analysis of cell division orientation in epidermal basal layer using intravital two-photon microscopy. PLoS One *11*, 1–18.

Islam, S., Kjällquist, U., Moliner, A., Zajac, P., Fan, J.B., Lönnerberg, P., and Linnarsson, S. (2011). Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. Genome Res. *21*, 1160–1167.

Islam, S., Zeisel, A., Joost, S., La Manno, G., Zajac, P., Kasper, M., Lönnerberg, P., and Linnarsson, S. (2014). Quantitative single-cell RNA-seq with unique molecular identifiers. Nat. Methods *11*, 163–166.

Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R.J., and Cotsarelis, G. (2005). Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat. Med. *11*, 1351–1354.

Ito, M., Yang, Z., Andl, T., Cui, C., Kim, N., Millar, S.E., and Cotsarelis, G. (2007). Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature *447*, 316–320.

Jahoda, C.A.B., Horne, K.A., and Oliver, R.F. (1984). Induction of hair growth by implantation of cultured dermal papilla cells. Nature *311*, 560–562.

Jaitin, D.A., Kenigsberg, E., Keren-Shaul, H., Elefant, N., Paul, F., Zaretsky, I., Mildner, A., Cohen, N., Jung, S., Tanay, A., et al. (2014). Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types. Science *343*, 776–779.

Jaks, V., Barker, N., Kasper, M., van Es, J.H., Snippert, H.J., Clevers, H., and Toftgård, R. (2008). Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat. Genet. *40*, 1291–1299.

Jaks, V., Kasper, M., and Toftgård, R. (2010). The hair follicle-a stem cell zoo. Exp. Cell Res. *316*, 1422–1428.

Jameson, J., Ugarte, K., Chen, N., Yachi, P., Fuchs, E., Boismenu, R., and Havran, W.L. (2002). A role for skin gamma delta T cells in wound repair. Science *296*, 747–749.

Jenkins, B.A., and Lumpkin, E.A. (2017). Developing a sense of touch. Dev. *144*, 4048–4090.

Jensen, K.B., Collins, C. a., Nascimento, E., Tan, D.W., Frye, M., Itami, S., and Watt, F.M. (2009). Lrig1 Expression Defines a Distinct Multipotent Stem Cell Population in Mammalian Epidermis. Cell Stem Cell *4*, 427–439.

Joost, S. (2019). Decoding the heterogeneity of skin in homeostasis and regeneration at single-cell resolution. Karolinska Institutet.

Joost, S., Zeisel, A., Jacob, T., Sun, X., La Manno, G., L??nnerberg, P., Linnarsson, S., and Kasper, M. (2016). Single-Cell Transcriptomics Reveals that Differentiation and Spatial Signatures Shape Epidermal and Hair Follicle Heterogeneity. Cell Syst. *3*, 221–237.

Kalhor, R., Kalhor, K., Mejia, L., Leeper, K., Graveline, A., Mali, P., and Church, G.M. (2018). Developmental barcoding of whole mouse via homing CRISPR. Science *361*.

Kaplan, D.H. (2017). Ontogeny and function of epidermal murine Langerhans cells. Nat Immunol. *18*, 1068–1075.

Karaiskos, N., Wahle, P., Alles, J., Boltengagen, A., Ayoub, S., Kipar, C., Kocks, C., Rajewsky, N., and Zinzen, R.P. (2017). The Drosophila embryo at single-cell transcriptome resolution. Science *358*, 194–199.

Kasper, M., Jaks, V., Are, A., Bergström, Å., Schwäger, A., Barker, N., and Toftgård, R. (2011). Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. Proc. Natl. Acad. Sci. USA *108*, 4099–4104.

Kasper, M., Jaks, V., Are, A., Bergström, Å., Schwäger, A., Svärd, J., and Teglund, S. (2012). Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. Proc. Natl. Acad. Sci. USA *109*, 5548–5548.

Kaushal, G.S., Rognoni, E., Lichtenberger, B.M., Driskell, R.R., Kretzschmar, K., Hoste, E., and Watt, F.M. (2015). Fate of Prominin-1 Expressing Dermal Papilla Cells during Homeostasis, Wound Healing and Wnt Activation. J. Invest. Dermatol. *135*, 2926–2934.

Ke, R., Mignardi, M., Pacureanu, A., Svedlund, J., Botling, J., Wählby, C., and Nilsson, M. (2013). In situ sequencing for RNA analysis in preserved tissue and cells. Nat. Methods *10*, 857–860.

Kern, D., Collins, M., Fultz, T., Detmer, J., Hamren, S., Peterkin, J.J., Sheridan, P., Urdea, M., White, R., Yeghiazarian, T., et al. (1996). An enhanced-sensitivity branched-DNA assay for quantification of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. *34*, 3196–3202.

Kester, L., and van Oudenaarden, A. (2018). Single-Cell Transcriptomics Meets Lineage Tracing. Cell Stem Cell *23*, 166–179.

Klein, A.M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D.A., and

Kirschner, M.W. (2015). Droplet barcoding for single cell transcriptomics applied to embryonic stem cells. Cell *161*, 1187–1201.

Kloepper, J.E., Kawai, K., Bertolini, M., Kanekura, T., and Paus, R. (2013). Loss of γδ t cells results in hair cycling defects. J. Invest. Dermatol. *133*, 1666–1669.

Kordon, E.C., and Smith, G.H. (1998). An entire functional mammary gland may comprise the progeny from a single cell. Development *125*, 1921–1930.

Koster, M.I., and Roop, D.R. (2007). Mechanisms regulating epithelial stratification. Annu. Rev. Cell Dev. Biol. *23*, 93–113.

Kretzschmar, K., and Watt, F.M. (2012). Lineage tracing. Cell *148*, 33–45.

Kumaravelu, P., Hook, L., Morrison, A.M., Ure, J., Zhao, S., Zuyev, S., Ansell, J., and Medvinsky, A. (2003). Quantitative developmental anatomy of definitive haematopoietic stem cells/ long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. Development *130*, 425.

Langton, A.K., Herrick, S.E., and Headon, D.J. (2008). An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. J. Invest. Dermatol. *128*, 1311–1318.

Larsson, C., Grundberg, I., Söderberg, O., and Nilsson, M. (2010). In situ detection and genotyping of individual mRNA molecules. Nat. Methods *7*, 395–397.

Larsson, L., Frisén, J., and Lundeberg, J. (2021). Spatially resolved transcriptomics adds a new dimension to genomics. Nat. Methods *18*, 15–18.

Lebonvallet, N., Laverdet, B., Misery, L., Desmoulière, A., and Girard, D. (2018). New insights into the roles of myofibroblasts and innervation during skin healing and innovative therapies to improve scar innervation. Exp. Dermatol. *27*, 950–958.

Lebre, M.C., Van Der Aar, A.M.G., Van Baarsen, L., Van Capel, T.M.M., Schuitemaker, J.H.N., Kapsenberg, M.L., and De Jong, E.C. (2007). Human keratinocytes express functional toll-like receptor 3, 4, 5, and 9. J. Invest. Dermatol. *127*, 331–341.

Lechler, T., and Fuchs, E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature *437*, 275–280.

Lee, J.H., Daugharthy, E.R., Scheiman, J., Kalhor, R., Amamoto, R., Peters, D.T., Turczyk, B.M., and Marblestone, A.H. (2014). Highly Multiplexed Subcellular RNA Sequencing in Situ. Science *343*, 1360–1364.

Legué, E., and Nicolas, J.-F. (2005). Hair follicle renewal: organization of stem cells in the matrix and the role of stereotyped lineages and behaviors. Development *132*, 4143–4154.

Legué, E., Sequeira, I., and Nicolas, J.-F. (2010). Hair follicle renewal: authentic morphogenesis that depends on a complex progression of stem cell lineages. Development *137*, 569–577.

Lein, E., Borm, L.E., and Linnarsson, S. (2017). The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing. Science *358*, 64–69.

Lesko, M.H., Driskell, R.R., Kretzschmar, K., Goldie, S.J., and Watt, F.M. (2013). Sox2 modulates the function of two distinct cell lineages in mouse skin. Dev. Biol. *382*, 15–26.

Levéen, P., Pekny, M., Gebre-Medhin, S., Swolin, B., Larsson, E., and Betsholtz, C. (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev. *8*, 1875–1887.

Li, K.N., Jain, P., He, C.H., Eun, F.C., Kang, S., and Tumbar, T. (2019). Skin vasculature and hair follicle cross-talking associated with stem cell activation and tissue homeostasis. Elife *8*, 1–27.

Li, W., Kohara, H., Uchida, Y., James, J.M., Soneji, K., Cronshaw, D.G., Zou, Y.R., Nagasawa, T., and Mukouyama, Y. suke (2013). Peripheral nerve-derived CXCL12 and VEGF-A regulate the patterning of arterial vessel branching in developing limb skin. Dev. Cell *24*, 359–371.

Lim, X., Tan, S.H.S.H., Koh, W.L.C., Chau, R.M.W., Yan, K.S., Kuo, C.J., van Amerongen, R., Klein, A.M., and Nusse, R. (2013). Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. Science *342*, 1226–1230.

Lin, J.Y., and Fisher, D.E. (2007). Melanocyte biology and skin pigmentation. Nature *445*, 843–850.

Lin, Z., Jin, S., Chen, J., Li, Z., Lin, Z., Tang, L., Nie, Q., and Andersen, B. (2020). Murine interfollicular epidermal differentiation is gradualistic with GRHL3 controlling progression from stem to transition cell states. Nat. Commun. *11*, 1–15.

Liu, N., Matsumura, H., Kato, T., Ichinose, S., Takada, A., Namiki, T., Asakawa, K., Morinaga, H., Mohri, Y., De Arcangelis, A., et al. (2019). Stem cell competition orchestrates skin homeostasis and ageing. Nature *568*, 344–350.

Livet, J., Weissman, T. a, Kang, H., Draft, R.W., Lu, J., Bennis, R. a, Sanes, J.R., and Lichtman, J.W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature *450*, 56–62.

Lombardi, T., Hauser, C., and Budtz-Jörgensen, E. (1993). Langerhans cells: structure, function and role in oral pathological conditions. J. Oral Pathol. Med. *22*, 193–202.

Lubeck, E., Coskun, A.F., Zhiyentayev, T., Ahmad, M., and Cai, L. (2014). Single-cell in situ RNA profiling by sequential hybridization. Nat. Methods *11*, 360–361.

Luecken, M.D., and Theis, F.J. (2019). Current best practices in single-cell RNA-seq analysis: a tutorial. Mol. Syst. Biol. *15*.

Lun, A.T.L., Bach, K., and Marioni, J.C. (2016). Pooling across cells to normalize single-cell RNA sequencing data with many zero counts. Genome Biol. *17*, 1–14.

Luttun, A., Tjwa, M., and Carmeliet, P. (2002). Placental Growth Factor (PIGF) and Its Receptor Flt-1 (VEGFR-1). Ann. N.Y. Acad. Sci. *979*, 80–93.

M'Boneko, V., and Merker, H.J. (1988). Development and morphology of the periderm of mouse embryos (days 9-12 of gestation). Acta Anat. (Basel). *133*, 325–336.

Ma, A., McDermaid, A., Xu, J., Chang, Y., and Ma, Q. (2020). Integrative Methods and Practical Challenges for Single-Cell Multi-omics. Trends Biotechnol. *38*, 1007–1022.

Ma, Q., Fode, C., Guillemot, F., and Anderson, D.J. (1999). Neurogenin1 and Neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. Genes Dev. *13*, 1717–1728.

van der Maaten, L., and Hinton, G. (2008). Visualizing Data using t-SNE. J. Mach. Learn. Res. *9*, 2579–2605.

Mackenzie, I.C. (1970). Relationship between Mitosis and the Ordered Structure of the Stratum Corneum in Mouse Epidermis. Nature *226*, 653–655.

Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell *161*, 1202–1214.

La Manno, G., Soldatov, R., Zeisel, A., Braun, E., Hochgerner, H., Petukhov, V., Lidschreiber, K., Kastriti, M.E., Lönnerberg, P., Furlan, A., et al. (2018). RNA velocity of single cells. Nature *560*, 494–498.

Martin, P. (1997). Wound healing-aiming for perfect skin regeneration. Science *276*, 75–81.

Martinez-Corral, I., Ulvmar, M.H., Stanczuk, L., Tatin, F., Kizhatil, K., John, S.W.M., Alitalo, K., Ortega, S., and Makinen, T. (2015). Nonvenous origin of dermal lymphatic vasculature. Circ. Res. *116*, 1649–1654.

Martino, P.A., Heitman, N., and Rendl, M. (2020). The dermal sheath: An emerging component of the hair follicle stem cell niche. Exp. Dermatol. *00*, 1–10.

Mascré, G., Dekoninck, S., Drogat, B., Youssef, K.K., Brohée, S., Sotiropoulou, P.A., Simons, B.D., Blanpain, C., Mascre, G., Dekoninck, S., et al. (2012). Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature *489*, 257–262.

Mass, E., Ballesteros, I., Farlik, M., Halbritter, F., Günther, P., Crozet, L., Jacome-Galarza, C.E., Händler, K., Klughammer, J., Kobayashi, Y., et al. (2016). Specification of tissue-resident macrophages during organogenesis. Science *353*.

Mathur, A.N., Zirak, B., Boothby, I.C., Tan, M., Cohen, J.N., Mauro, T.M., Mehta, P., Lowe, M.M., Abbas, A.K., Ali, N., et al. (2019). Treg-Cell Control of a CXCL5-IL-17 Inflammatory Axis Promotes Hair-Follicle-Stem-Cell Differentiation During Skin-Barrier Repair. Immunity *50*, 655-667.e4.

Matos, I., Asare, A., Levorse, J., Ouspenskaia, T., de la Cruz-Racelis, J., Schuhmacher, L.N., and Fuchs, E.

(2020). Progenitors oppositely polarize wnt activators and inhibitors to orchestrate tissue development. Elife *9*, 1–22.

Matsumura, H., Mohri, Y., Binh, N.T., Morinaga, H., Fukuda, M., Ito, M., Kurata, S., Hoeijmakers, J., and Nishimura, E.K. (2016). Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. Science *351*, 1–14.

Mayr, U., Serra, D., and Liberali, P. (2019). Exploring single cells in space and time during tissue development, homeostasis and regeneration. Dev. *146*.

McInnes, L., Healy, J., Saul, N., and Großberger, L. (2018). UMAP: Uniform Manifold Approximation and Projection. J. Open Source Softw. *3*, 861.

McKenna, A., and Gagnon, J.A. (2019). Recording development with single cell dynamic lineage tracing. Dev. *146*, 1–10.

Mecklenburg, L., Tobin, D.J., Müller-Röver, S., Handjiski, B., Wendt, G., Peters, E.M.J., Pohl, S., Moll, I., and Paus, R. (2000). Active hair growth (anagen) is associated with angiogenesis. J. Invest. Dermatol. *114*, 909–916.

Medvinsky, A., and Dzierzak, E. (1996). Definitive hematopoiesis is autonomously initiated by the AGM region. Cell *86*, 897–906.

Mesa, K.R., Rompolas, P., and Greco, V. (2015a). The Dynamic Duo: Niche/Stem Cell Interdependency. Stem Cell Reports *4*, 961–966.

Mesa, K.R., Rompolas, P., Zito, G., Myung, P., Sun, T.Y., Brown, S., Gonzalez, D.G., Blagoev, K.B., Haberman, A.M., and Greco, V. (2015b). Niche-induced cell death and epithelial phagocytosis regulate hair follicle stem cell pool. Nature *522*, 94–97.

Mesa, K.R., Kawaguchi, K., Cockburn, K., Gonzalez, D., Boucher, J., Xin, T., Klein, A.M., and Greco, V. (2018). Homeostatic Epidermal Stem Cell Self-Renewal Is Driven by Local Differentiation. Cell Stem Cell *23*, 677-686.e4.

Mesler, A.L., Veniaminova, N.A., Lull, M. V., Wong, S.Y., Madison, V.L., and Wong, S.Y. (2017). Hair Follicle Terminal Differentiation Is Orchestrated by Distinct Early and Late Matrix Progenitors. Cell Rep. *19*, 809–821.

Millar, S.E. (2002). Molecular mechanisms regulating hair follicle development. J. Invest. Dermatol. *118*, 216–225.

Miller, L.S., and Modlin, R.L. (2007). Human keratinocyte Toll-like receptors promote distinct immune responses. J. Invest. Dermatol. *127*, 262–263.

Miroshnikova, Y.A., Le, H.Q., Schneider, D., Thalheim, T., Rübsam, M., Bremicker, N., Polleux, J., Kamprad, N., Tarantola, M., Wang, I., et al. (2018). Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. Nat. Cell Biol. *20*, 69–80.

Moffitt, J.R., Hao, J., Bambah-Mukku, D., Lu, T., Dulac, C., and Zhuang, X. (2016). High-performance multiplexed fluorescence in situ hybridization in culture and tissue with matrix imprinting and clearing. Proc. Natl. Acad. Sci. USA *113*, 14456–14461.

Mok, K.W., Saxena, N., Heitman, N., Grisanti, L., Srivastava, D., Muraro, M.J., Jacob, T., Sennett, R., Wang, Z., Su, Y., et al. (2019). Dermal Condensate Niche Fate Specification Occurs Prior to Formation and Is Placode Progenitor Dependent. Dev. Cell *48*, 32-48.e5.

Moore, M.A.S., and Metcalf, D. (1970). Ontogeny of the Haemopoietic System: Yolk Sac Origin of In Vivo and In Vitro Colony Forming Cells in the Developing Mouse Embryo. Br. J. Haematol. *18*, 279–296.

Morita, H., Saito, H., Matsumoto, K., and Nakae, S. (2016). Regulatory roles of mast cells in immune responses. Semin. Immunopathol. *38*, 623–629.

Morris, R.J., Liu, Y., Marles, L., Yang, Z., Trempus, C., Li, S., Lin, J.S., Sawicki, J.A., and Cotsarelis, G. (2004). Capturing and profiling adult hair follicle stem cells. Nat. Biotechnol. *22*, 411–417.

Morrison, K.M., Miesegaes, G.R., Lumpkin, E. a., and Maricich, S.M. (2009). Mammalian Merkel cells are descended from the epidermal lineage. Dev. Biol. *336*, 76–83.

Mukouyama, Y., Shin, D., Britsch, S., Taniguchi, M., and Anderson, D.J. (2002). Sensory Nerves Determine the Pattern of Arterial Differentiation and Blood Vessel Branching in the Skin. Cell *109*, 693–705.

Müller-Röver, S., Handjiski, B., Van Der Veen, C., Eichmüller, S., Foitzik, K., McKay, I. a., Stenn, K.S., and Paus, R. (2001). A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. J. Invest. Dermatol. *117*, 3–15.

Naito, M., Takahashi, K., and Nishikawa, S. (1990). Development, Differentiation, and Maturation of Macrophages in the Fetal Mouse Liver. J. Leukoc. Biol. *48*, 27–37.

Närhi, K., Järvinen, E., Birchmeier, W., Taketo, M.M., Mikkola, M.L., Thesleff, I., Narhi, K., Jarvinen, E., Birchmeier, W., Taketo, M.M., et al. (2008). Sustained epithelial b-catenin activity induces precocious hair development but disrupts hair follicle down-growth and hair shaft formation. Development *135*, 1019–1028.

Nehls, V., and Drenckhahn, D. (1993). The versatility of microvascular pericytes: from mesenchyme to smooth muscle? Histochemistry *99*, 1–12.

Newman, A.C., Nakatsu, M.N., Chou, W., Gershon, P.D., and Hughes, C.C.W. (2011). The requirement for fibroblasts in angiogenesis: Fibroblast-derived matrix proteins are essential for endothelial cell lumen formation. Mol. Biol. Cell *22*, 3791–3800.

Nguyen, H., Rendl, M., and Fuchs, E. (2006). Tcf3 Governs Stem Cell Features and Represses Cell Fate Determination in Skin. Cell *127*, 171–183.

Niemann, C., and Horsley, V. (2012). Development and homeostasis of the sebaceous gland. Semin. Cell Dev. Biol. *23*, 928–936.

Nilsson, M., Malmgren, H., Samiotaki, M., Kwiatkowski, M., Chowdhary, B.P., and Landegren, U. (1994). Padlock probes: Circularizing oligonucleotides for localized DNA detection. Science *265*, 2085–2088.

Nishimura, E.K., Jordan, S.A., Oshima, H., Yoshida, H., Osawa, M., Moriyama, M., Jackson, I.J., Barrandon, Y., Miyachi, Y., and Nishikawa, S.-I. (2002). Dominant role of the niche in melanocyte stem-cell fate determination. Nature *416*, 854–860.

Nitzan, M., Karaiskos, N., Friedman, N., and Rajewsky, N. (2019). Gene expression cartography. Nature *576*, 132–137.

Noramly, S., and Morgan, B.A. (1998). BMPs mediate lateral inhibition at successive stages in feather tract development. Development *125*, 3775–3787.

Nowak, J. a., Polak, L., Pasolli, H.A., and Fuchs, E. (2008). Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell *3*, 33–43.

Oikonomopoulos, S., Wang, Y.C., Djambazian, H., Badescu, D., and Ragoussis, J. (2016). Benchmarking of the Oxford Nanopore MinION sequencing for quantitative and qualitative assessment of cDNA populations. Sci. Rep. *6*, 1–13.

Orkin, S.H., and Zon, L.I. (2008). Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. Cell *132*, 631–644.

Osaka, N., Takahashi, T., Murakami, S., Matsuzawa, A., Noguchi, T., Fujiwara, T., Aburatani, H., Moriyama, K., Takeda, K., and Ichijo, H. (2007). ASK1-dependent recruitment and activation of macrophages induce hair growth in skin wounds. J. Cell Biol. *176*, 903–909.

Oshimori, N., and Fuchs, E. (2012). Paracrine TGF-β signaling counterbalances BMP-mediated repression in hair follicle stem cell activation. Cell Stem Cell *10*, 63–75.

Ostrowski, S.M., Wright, M.C., Bolock, A.M., Geng, X., and Maricich, S.M. (2015). Ectopic Atoh1 expression drives Merkel cell production in embryonic, postnatal and adult mouse epidermis. Dev. *142*, 2533–2544.

Ouspenskaia, T., Matos, I., Mertz, A.F., Fiore, V.F., and Fuchs, E. (2016). WNT-SHH Antagonism Specifies and Expands Stem Cells prior to Niche Formation. Cell *164*, 156–169.

Page, M.E., Lombard, P., Ng, F., Göttgens, B., and Jensen, K.B. (2013). The epidermis comprises autonomous compartments maintained by distinct stem cell populations. Cell Stem Cell *13*, 471–482.

Palacios, R., and Imhof, B.A. (1993). At day 8-8.5 of mouse development the yolk sac, not the embryo proper, has lymphoid precursor potential in vivo and in vitro. Proc. Natl. Acad. Sci. USA *90*, 6581–6585.

Palazzo, E., Marconi, A., Truzzi, F., Dallaglio, K., Petrachi, T., Humbert, P., Schnebert, S., Perrier, E., Dumas, M., and Pincelli, C. (2012). Role of neurotrophins on dermal fibroblast survival and differentiation. J. Cell. Physiol. *227*, 1017–1025.

Park, A.S., Matte-martone, C., Gonzalez, D.G., Lathrop, E.A., May, D.P., Pineda, C.M., Moore, J.L., Boucher, J.D., and Marsh, E.D. (2021). Skin-resident immune cells actively coordinate their distributino with epidermal cells during homeostasis. BioRxiv.

Park, S., Gonzalez, D.G., Guirao, B., Boucher, J.D., Cockburn, K., Marsh, E.D., Mesa, K.R., Brown, S., Rompolas, P., Haberman, A.M., et al. (2017). Tissue-scale coordination of cellular behaviour promotes epidermal wound repair in live mice. Nat. Cell Biol. *19*, 155–163.

Pasolli, H.A. (2011). The hair follicle bulge: a niche for adult stem cells. Microsc. Microanal. *17*, 513–519.

Paus, R. (1998). Principles of hair cycle control. J. Dermatol. *25*, 793–802.

Paus, R., Maurer, M., Slominski, A., and Czarnetzki, B.M. (1994). Mast Cell Involvement in Murine Hair Growth. Dev. Biol. *143*, 230–240.

Paus, R., Müller-Röver, S., Van Der Veen, C., Maurer, M., Eichmüller, S., Ling, G., Hofmann, U., Foitzik, K., Mecklenburg, L., and Handjiski, B. (1999). A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. J. Invest. Dermatol. *113*, 523–532.

Peña-Jimenez, D., Fontenete, S., Megias, D., Fustero-Torre, C., Graña-Castro, O., Castellana, D., Loewe, R., and Perez-Moreno, M. (2019). Lymphatic vessels interact dynamically with the hair follicle stem cell niche during skin regeneration in vivo . EMBO J. *38*, 1–17.

Petruska, J.C., Barker, D.F., Garraway, S.M., Trainer, R., Fransen, J.W., Seidman, P.A., Soto, R.G., Mendell, L.M., and Johnson, R.D. (2014). Organization of sensory input to the nociceptive-specific cutaneous trunk muscle reflex in rat, an effective experimental system for examining nociception and plasticity. J Comp Neurol *522*, 1048–1071.

Philippeos, C., Telerman, S.B., Oulès, B., Pisco, A.O., Shaw, T.J., Elgueta, R., Lombardi, G., Driskell, R.R., Soldin, M., Lynch, M.D., et al. (2018). Spatial and Single-Cell Transcriptional Profiling Identifies Functionally Distinct Human Dermal Fibroblast Subpopulations. J. Invest. Dermatol. *138*, 811–825.

Picelli, S., Faridani, O.R., Björklund, A.K., Winberg, G., Sagasser, S., and Sandberg, R. (2014). Full-length RNA-seq from single cells using Smart-seq2. Nat. Protoc. *9*, 171–181.

Pichon, X., Lagha, M., Mueller, F., and Bertrand, E. (2018). A Growing Toolbox to Image Gene Expression in Single Cells: Sensitive Approaches for Demanding Challenges. Mol. Cell *71*, 468–480.

Pineda, C.M., Park, S., Mesa, K.R., Wolfel, M., Gonzalez, D.G., Haberman, A.M., Rompolas, P., and Greco, V. (2015). Intravital imaging of hair follicle regeneration in the mouse. Nat. Protoc. *10*, 1116–1130.

Player, A.N., Shen, L.P., Kenny, D., Antao, V.P., and Kolberg, J.A. (2001). Single-copy gene detection using branched DNA (bDNA) in situ hybridization. J. Histochem. Cytochem. *49*, 603–611.

Plikus, M. V., and Chuong, C.M. (2014). Macroenvironmental regulation of hair cycling and collective regenerative behavior. Cold Spring Harb. Perspect. Med. *4*, 1–14.

Plikus, M. V., Guerrero-Juarez, C.F., Ito, M., Li, Y.R., Dedhia, P.H., Zheng, Y., Shao, M., Gay, D.L., Ramos, R., Hsi, T.-C., et al. (2017). Regeneration of fat cells from myofibroblasts during wound healing. Science *355*, 748–752.

Plikus, M. V, Mayer, J.A., de la Cruz, D., Baker, R.E., Maini, P.K., Maxson, R., and Chuong, C.-M. (2008). Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. Nature *451*, 340–344.

Polański, K., Young, M.D., Miao, Z., Meyer, K.B., Teichmann, S.A., and Park, J.E. (2020). BBKNN: Fast batch alignment of single cell transcriptomes. Bioinformatics *36*, 964–965.

Potten, C.S. (1974). The Epidermal Proliferative Unit: The Possible Role of the Central Basal Cell. Cell Tissue Kinet. *7*, 77–88.

Potten, C.S., and Morris, R.J. (1988). Epithelial stem cells in vivo. J. Cell Sci. Suppl. *10*, 45–62.

Rahmani, W., Abbasi, S., Hagner, A., Raharjo, E., Kumar, R., Hotta, A., Magness, S., Metzger, D., and Biernaskie, J. (2014). Hair Follicle Dermal Stem Cells Regenerate the Dermal Sheath, Repopulate the Dermal Papilla, and Modulate Hair Type. Dev. Cell *31*, 543–558.

Rahmani, W., Sinha, S., and Biernaskie, J. (2020). Immune modulation of hair follicle regeneration. Npj Regen. Med. *5*, 1–13.

Ramsköld, D., Luo, S., Wang, Y.-C., Li, R., Deng, Q., Faridani, O.R., Daniels, G.A., Khrebtukova, I., Loring, J.F., Laurent, L.C., et al. (2012). Full-Length mRNA-Seq from single cell levels of RNA and individual circulating tumor cells. Nat. Biotechnol. *30*, 777–782.

Regev, A., Teichmann, S.A., Lander, E.S., Amit, I., Benoist, C., Birney, E., Bodenmiller, B., Campbell, P., Carninci, P., Clatworthy, M., et al. (2017). The human cell atlas. Elife *6*, 1–30.

Rezza, A., Wang, Z., Sennett, R., Qiao, W., Wang, D., Heitman, N., Mok, K.W., Clavel, C., Yi, R., Zandstra, P., et al. (2016). Signaling Networks among Stem Cell Precursors, Transit-Amplifying Progenitors, and their Niche in Developing Hair Follicles. Cell Rep. *14*, 3001–3018.

Rhee, H., Polak, L., and Fuchs, E. (2006). Lhx2 maintains stem cell character in hair follicles. Science *312*, 1946–1949.

Ribatti, D., and Santoiemma, M. (2014). Epithelial-mesenchymal interactions: A fundamental developmental biology mechanism. Int. J. Dev. Biol. *58*, 303–306.

Ribatti, D., Vacca, A., and Presta, M. (2000). The discovery of angiogenic factors: A historical review. Gen. Pharmacol. Vasc. Syst. *35*, 227–231.

Richardson, R.J., Hammond, N.L., Coulombe, P.A., Saloranta, C., Nousiainen, H.O., Salonen, R., Berry, A., Hanley, N., Headon, D., Karikoski, R., et al. (2014). Periderm prevents pathological epithelial adhesions during embryogenesis. J. Clin. Invest. *124*, 3891–3900.

Rinkevich, Y., Walmsley, G.G., Hu, M.S., Maan, Z.N., Newman, A.M., Drukker, M., Januszyk, M., Krampitz, G.W., Gurtner, G.C., Lorenz, H.P., et al. (2015). Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science *348*.

Rivas, R.J., Burmeister, D.W., and Goldberg, D.J. (1992). Rapid effects of laminin on the growth cone. Neuron *8*, 107–115.

Rivera-Gonzalez, G.C., Shook, B.A., Andrae, J., Holtrup, B., Bollag, K., Betsholtz, C., Rodeheffer, M.S., and Horsley, V. (2016). Skin Adipocyte Stem Cell Self-Renewal Is Regulated by a PDGFA/AKT-Signaling Axis. Cell Stem Cell *19*, 738–751.

Rochat, A., Kobayashi, K., and Barrandon, Y. (1994). Location of stem cells of human hair follicles by clonal analysis. Cell *76*, 1063–1073.

Rodriques, S.G., Stickels, R.R., Goeva, A., Martin, C.A., Murray, E., Vanderburg, C.R., Welch, J., Chen, L.M., Chen, F., and Macosko, E.Z. (2019). Slide-seq: A Scalable Technology for Measuring Genome-Wide Expression at High Spatial Resolution. Science *363*, 1463–1467.

Rognoni, E., and Watt, F.M. (2018). Skin Cell Heterogeneity in Development, Wound Healing, and Cancer. Trends Cell Biol. *28*, 709–722.

Rognoni, E., Gomez, C., Pisco, A.O., Rawlins, E.L., Simons, B.D., Watt, F.M., and Driskell, R.R. (2016). Inhibition of β-catenin signalling in dermal fibroblasts enhances hair follicle regeneration during wound healing. Development *143*, 2522–2535.

Rognoni, E., Pisco, A.O., Hiratsuka, T., Sipilä, K.H., Belmonte, J.M., Mobasseri, S.A., Philippeos, C., Dilão, R., and Watt, F.M. (2018). Fibroblast state switching orchestrates dermal maturation and wound healing. Mol. Syst. Biol. *14*, 1–20.

Romani, B.N., Koide, S., Crowley, M., Pack, M.W., Livingstone, A.M., Fathman, C.G., Inaba, K., and Steinman, R.M. (1989). Presentation of exogenous protein antigens by dendritic cells to T cell clones. J. Exp. Med. *169*, 1169–1178.

Romani, N., Schuler, G., and Fritsch, P. (1986). Ontogeny of Ia-positive and Thy-1-positive leukocytes of murine epidermis. J. Invest. Dermatol. *86*, 129–133.

Rompolas, P., Deschene, E.R., Zito, G., Gonzalez, D.G., Saotome, I., Haberman, A.M., and Greco, V. (2012). Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. Nature *487*, 496–499.

Rompolas, P., Mesa, K.R., and Greco, V. (2013). Spatial organization within a niche as a determinant of stem-cell fate. Nature *502*, 513–518.

Rosenberg, A.B., Roco, C.M., Muscat, R.A., Kuchina, A., Sample, P., Yao, Z., Graybuck, L.T., Peeler, D.J., Mukherjee, S., Chen, W., et al. (2018). Single-cell profiling of the developing mouse brain and spinal cord with

split-pool barcoding. Science *360*, 176–182.

Rouhanifard, S.H., Mellis, I.A., Dunagin, M., Bayatpour, S., Jiang, C.L., Dardani, I., Symmons, O., Emert, B., Torre, E., Cote, A., et al. (2019). ClampFISH detects individual nucleic acid molecules using click chemistry–based amplification. Nat. Biotechnol. *37*, 84–94.

Ruchti, C., Haller, D., Nuber, M., and Cottier, H. (1983). Regional differences in renewal rates of fibroblasts in young adult female mice. Cell Tissue Res. *232*, 625–636.

Ruhrberg, C., Gerhardt, H., Golding, M., Watson, R., Ioannidou, S., Fujisawa, H., Betsholtz, C., and Shima, D.T. (2002). Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev. *16*, 2684–2698.

Sada, A., Jacob, F., Leung, E., Wang, S., White, B.S., Shalloway, D., and Tumbar, T. (2016). Defining the cellular lineage hierarchy in the interfollicular epidermis of adult skin. Nat. Cell Biol. *18*, 619–631.

Saelens, W., Cannoodt, R., Todorov, H., and Saeys, Y. (2019). A comparison of single-cell trajectory inference methods. Nat. Biotechnol. *37*, 547–554.

Salzer, M.C., Lafzi, A., Berenguer-Llergo, A., Youssif, C., Castellanos, A., Solanas, G., Peixoto, F.O., Stephan-Otto Attolini, C., Prats, N., Aguilera, M., et al. (2018). Identity Noise and Adipogenic Traits Characterize Dermal Fibroblast Aging. Cell *175*, 1575-1590.e22.

Sarin, K.Y., and Artandi, S.E. (2007). Aging, graying and loss of melanocyte stem cells. Stem Cell Rev. *3*, 212–217.

Satija, R., Farrell, J.A., Gennert, D., Schier, A.F., and Regev, A. (2015). Spatial reconstruction of single-cell gene expression data. Nat. Biotechnol. *33*, 495–502.

Sauer, B. (1998). Inducible gene targeting in mice using the Cre/lox system. Methods *14*, 381–392.

Saxena, N., Mok, K.W., and Rendl, M. (2019). An updated classification of hair follicle morphogenesis. Exp. Dermatol. *28*, 332–344.

Scharschmidt, T.C., Vasquez, K.S., Pauli, M.L., Leitner, E.G., Chu, K., Truong, H., Lowe, M.M., Rodriguez, R.S., Ali, N., Laszik, Z.G., et al. (2017). Commensal Microbes and Hair Follicle Morphogenesis Coordinately Drive Treg Migration into Neonatal Skin. Cell Host Microb *21*, 467–477.

Schaum, N., Karkanias, J., Neff, N.F., May, A.P., Quake, S.R., Wyss-Coray, T., Darmanis, S., Batson, J., Botvinnik, O., Chen, M.B., et al. (2018). Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. Nature *562*, 367–372.

Schepeler, T., Page, M.E., and Jensen, K.B. (2014). Heterogeneity and plasticity of epidermal stem cells. Development *141*, 2559–2567.

Schlake, T. (2007). Determination of hair structure and shape. Semin. Cell Dev. Biol. *18*, 267–273.

Schmidt-Ullrich, R., and Paus, R. (2005). Molecular principles of hair follicle induction and morphogenesis. BioEssays *27*, 247–261.

Schmidt, B.A., and Horsley, V. (2013). Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development *140*, 1517–1527.

Schneider, M.R., Schmidt-Ullrich, R., and Paus, R. (2009). The Hair Follicle as a Dynamic Miniorgan. Curr. Biol. *19*, 132–142.

Schulte-Merker, S., Sabine, A., and Petrova, T. V. (2011). Lymphatic vascular morphogenesis in development, physiology, and disease. J. Cell Biol. *193*, 607–618.

Schweizer, J., Kinjo, M., Fürstenberger, G., and Winter, H. (1984). Sequential expression of mRNA-encoded keratin sets in neonatal mouse epidermis: Basal cells with properties of terminally differentiating cells. Cell *37*, 159–170.

Scott, J.R., Muangman, P., and Gibran, N.S. (2007). Making sense of hypertrophic scar: A role for nerves. Wound Repair Regen. *15*, 10–13.

Scudamore, C.L., Centre, M.L., and Harwell, M.R.C. (2014). Chapter 10 Integument and adipose tissue. In A Practical Guide to the Histology of the Mouse, (John Wiley & Sons, Ltd.), pp. 169–177.

Sennett, R., and Rendl, M. (2012). Mesenchymal-epithelial interactions during hair follicle morphogenesis and

cycling. Semin. Cell Dev. Biol. *23*, 917–927.

Sennett, R., and Rendl, M. (2015). A scar is born: origins of fibrotic skin tissue. Science *348*, 284–285.

Sequeira, I., and Nicolas, J.-F. (2012). Redefining the structure of the hair follicle by 3D clonal analysis. Development *139*, 3741–3751.

Shah, S., Lubeck, E., Schwarzkopf, M., He, T.F., Greenbaum, A., Sohn, C. ho, Lignell, A., Choi, H.M.T.T., Gradinaru, V., Pierce, N.A., et al. (2016). Single-molecule RNA detection at depth by hybridization chain reaction and tissue hydrogel embedding and clearing. Dev. *143*, 2862–2867.

Sharon, D., Tilgner, H., Grubert, F., and Snyder, M. (2013). A single-molecule long-read survey of the human transcriptome. Nat. Biotechnol. *31*, 1009–1014.

Shin, W., Rosin, N.L., Sparks, H., Sinha, S., Rahmani, W., Sharma, N., Workentine, M., Abbasi, S., Labit, E., Stratton, J.A., et al. (2020). Dysfunction of Hair Follicle Mesenchymal Progenitors Contributes to Age-Associated Hair Loss. Dev. Cell *53*, 185-198.e7.

Shook, B.A., Wasko, R.R., Mano, O., Rutenberg-Schoenberg, M., Rudolph, M.C., Zirak, B., Rivera-Gonzalez, G.C., López-Giráldez, F., Zarini, S., Rezza, A., et al. (2020). Dermal Adipocyte Lipolysis and Myofibroblast Conversion Are Required for Efficient Skin Repair. Cell Stem Cell *26*, 880-895.e6.

Shwartz, Y., Gonzalez-Celeiro, M., Chen, C.L., Pasolli, H.A., Sheu, S.H., Fan, S.M.Y., Shamsi, F., Assaad, S., Lin, E.T.Y., Zhang, B., et al. (2020). Cell Types Promoting Goosebumps Form a Niche to Regulate Hair Follicle Stem Cells. Cell *182*, 578-593.e19.

Shyer, A.E., Rodrigues, A.R., Schroeder, G.G., Kassianidou, E., Kumar, S., and Harland, R.M. (2017). Emergent cellular self-organization and mechanosensation initiate follicle pattern in the avian skin. Science *357*, 811–815.

Sick, S., Reinker, S., Timmer, J., and Schlake, T. (2006). WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. Science *314*, 1447–1450.

Sidgwick, G.P., and Bayat, A. (2012). Extracellular matrix molecules implicated in hypertrophic and keloid scarring. J. Eur. Acad. Dermatology Venereol. *26*, 141–152.

Skobe, M., and Detmar, M. (2000). Structure, function, and molecular control of the skin lymphatic system. J. Investig. Dermatology Symp. Proc. *5*, 14–19.

Smith, G.H., and Medina, D. (1988). A morphologically distinct candidate for an epithelial stem cell in mouse mammary gland. J. Cell Sci. *90*, 173–183.

Smith, P.G., and Liu, M. (2002). Impaired cutaneous wound healing after sensory denervation in developing rats: Effects on cell proliferation and apoptosis. Cell Tissue Res. *307*, 281–291.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D., et al. (2010a). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell *143*, 134–144.

Snippert, H.J., Haegebarth, A., Kasper, M., Jaks, V., van Es, J.H., Barker, N., van de Wetering, M., van den Born, M., Begthel, H., Vries, R.G., et al. (2010b). Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science *327*, 1385–1389.

Soriano, P. (1994). Abnormal kidney development and hematological disorders in PDGF β- receptor mutant mice. Genes Dev. *8*, 1888–1896.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. *21*, 70–71.

Sountoulidis, A., Liontos, A., Nguyen, H.P., Firsova, A.B., Fysikopoulos, A., Qian, X., Seeger, W., Sundström, E., Nilsson, M., and Samakovlis, C. (2020). SCRINSHOT enables spatial mapping of cell states in tissue sections with single-cell resolution. PLoS Biol. *18*, 1–32.

Ståhl, P.L., Salmén, F., Vickovic, S., Lundmark, A., Navarro, J.F., Magnusson, J., Giacomello, S., Asp, M., Westholm, J.O., Huss, M., et al. (2016). Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science *353*, 78–82.

Stifani, N. (2014). Motor neurons and the generation of spinal motor neuron diversity. Front. Cell. Neurosci. *8*, 1–22.

Su, Q., and Igyártó, B.Z. (2019). Keratinocytes Share Gene Expression Fingerprint with Epidermal Langerhans Cells via mRNA Transfer. J. Invest. Dermatol. *139*, 2313-2323.e8.

Sundberg, J.P., Peters, E.M.J., and Paus, R. (2005). Analysis of hair follicles in mutant laboratory mice. J. Investig. Dermatol. Symp. Proc. *10*, 264–270.

Suzuki, S., Kato, T., Takimoto, H., Masui, S., Oshima, H., Ozawa, K., Suzuki, S., and Imamura, T. (1998). Localization of rat FGF-5 protein in skin macrophage-like cells and FGF-5S protein in hair follicle: Possible involvement of two Fgf-5 gene products in hair growth cycle regulation. J. Invest. Dermatol. *111*, 963–972.

Szeder, V., Grim, M., Halata, Z., and Sieber-Blum, M. (2003). Neural crest origin of mammalian Merkel cells. Dev. Biol. *253*, 258–263.

Tan, S.Y., Roediger, B., and Weninger, W. (2015). The role of chemokines in cutaneous immunosurveillance. Immunol. Cell Biol. *93*, 337–346.

Tanay, A., and Regev, A. (2017). Scaling single-cell genomics from phenomenology to mechanism. Nature *541*, 331–338.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., et al. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. Nat. Methods *6*, 377–382.

Tanimura, S., Tadokoro, Y., Inomata, K., Binh, N.T., Nishie, W., Yamazaki, S., Nakauchi, H., Tanaka, Y., McMillan, J.R., Sawamura, D., et al. (2011). Hair follicle stem cells provide a functional niche for melanocyte stem cells. Cell Stem Cell *8*, 177–187.

Telerman, S.B., Rognoni, E., Sequeira, I., Pisco, A.O., Lichtenberger, B.M., Culley, O.J., Viswanathan, P., Driskell, R.R., and Watt, F.M. (2017). Dermal Blimp1 acts downstream of epidermal TGFβ and Wnt/β-catenin to regulate hair follicle formation and growth. J. Invest. Dermatol. *137*, 2270–2281.

Temple, S. (1989). Division and differentiation of isolated CNS blast cells in microculture. Nature *340*, 471–473.

Thelen, F., and Witherden, D.A. (2020). Get in Touch With Dendritic Epithelial T Cells! Front. Immunol. *11*, 1–11.

Theriault, E., and Diamond, J. (1988). Nociceptive cutaneous stimuli evoke localized contractions in a skeletal muscle. J. Neurophysiol. *60*, 446–462.

Till, J.E., and McCulloch, E.A. (2012). A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells. Radiat. Res. *178*.

Tobin, D.J., Slominski, A., Botchkarev, V., and Paus, R. (1999). The Fate of Hair Follicle Melanocytes During the Hair Growth Cycle. J. Investig. Dermatology Symp. Proc. *4*, 323–332.

Tong, P.L., Roediger, B., Kolesnikoff, N., Biro, M., Tay, S.S., Jain, R., Shaw, L.E., Grimbaldeston, M.A., and Weninger, W. (2015). The skin immune atlas: three-dimensional analysis of cutaneous leukocyte subsets by multiphoton microscopy. J. Invest. Dermatol. *135*, 84–93.

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., Lennon, N.J., Livak, K.J., Mikkelsen, T.S., and Rinn, J.L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. Nat. Biotechnol. *32*, 381–386.

Trempus, C.S., Morris, R.J., Bortner, C.D., Cotsarelis, G., Faircloth, R.S., Reece, J.M., and Tennant, R.W. (2003). Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. J. Invest. Dermatol. *120*, 501–511.

Tripp, C.H., Chang-Rodriguez, S., Stoitzner, P., Holzmann, S., Stössel, H., Douillard, P., Saeland, S., Koch, F., Elbe-Bürger, A., and Romani, N. (2004). Ontogeny of langerin/CD207 expression in the epidermis of mice. J. Invest. Dermatol. *122*, 670–672.

Tumbar, T., Guasch, G.G., Greco, V., Blanpain, C.C.C., Lowry, W.E., Rendl, M., and Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. Science *303*, 359–363.

Turing, A.M. (1952). The chemical basis of morphogenesis. Bull. Math. Biol. *52*, 37–72.

Vetrugno, R., Liguori, R., Cortelli, P., and Montagna, P. (2003). Sympathetic skin response: Basic mechanisms and clinical applications. Clin. Auton. Res. *13*, 256–270.

Vickovic, S., Eraslan, G., Salmén, F., Klughammer, J., Stenbeck, L., Schapiro, D., Äijö, T., Bonneau, R.,

Bergenstråhle, L., Navarro, J.F., et al. (2019). High-definition spatial transcriptomics for in situ tissue profiling. Nat. Methods *16*, 987–990.

Vidal, V.P.I., Chaboissier, M.C., Lützkendorf, S., Cotsarelis, G., Mill, P., Hui, C.C., Ortonne, N., Ortonne, J.P., and Schedl, A. (2005). Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. Curr. Biol. *15*, 1340–1351.

Wagner, A., Regev, A., and Yosef, N. (2016). Revealing the vectors of cellular identity with single-cell genomics. Nat. Biotechnol. *34*, 1145–1160.

Wang, E.C.E., Dai, Z., Ferrante, A.W., Drake, C.G., and Christiano, A.M. (2019). A Subset of TREM2 + Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth. Cell Stem Cell *24*, 654-669.e6.

Wang, F., Flanagan, J., Su, N., Wang, L.C., Bui, S., Nielson, A., Wu, X., Vo, H.T., Ma, X.J., and Luo, Y. (2012). RNAscope: A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J. Mol. Diagnostics *14*, 22–29.

Wang, X., Chen, H., Tian, R., Zhang, Y., Drutskaya, M.S., Wang, C., Ge, J., Fan, Z., Kong, D., Wang, X., et al. (2017). Macrophages induce AKT/β-catenin-dependent Lgr5+ stem cell activation and hair follicle regeneration through TNF. Nat. Commun. *8*, 1–14.

Wang, X., Allen, W.E., Wright, M.A., Sylwestrak, E.L., Samusik, N., Vesuna, S., Evans, K., Liu, C., Ramakrishnan, C., Liu, J., et al. (2018). Three-dimensional intact-tissue sequencing of single-cell transcriptional states. Science *361*.

Watt, F.M. (2016). Engineered Microenvironments to Direct Epidermal Stem Cell Behavior at Single-Cell Resolution. Dev. Cell *38*, 601–609.

Watt, F.M., and Fujiwara, H. (2011). Cell-extracellular matrix interactions in normal and diseased skin. Cold Spring Harb. Perspect. Biol. *3*, 1–14.

Watt, F.M., and Green, H. (1982). Stratification and terminal differentiation of cultured epidermal cells. Nature *295*, 434–436.

Weiss, L.W., and Zelickson, A.S. (1975). Embryology of the epidermis: ultra-structural aspects. III. Maturation and primary appearance of dendritic cells in the mouse with mammalian comparisons. Acta Derm. Venereol. *55*, 431–442.

Werner, S., Krieg, T., and Smola, H. (2007). Keratinocyte-fibroblast interactions in wound healing. J. Invest. Dermatol. *127*, 998–1008.

Weston, J.A. (1970). The migration and differentiation of neural crest cells. Adv Morphog *8*, 41–114.

Wojciechowicz, K., Gledhill, K., Ambler, C.A., Manning, C.B., and Jahoda, C.A.B. (2013). Development of the Mouse Dermal Adipose Layer Occurs Independently of Subcutaneous Adipose Tissue and Is Marked by Restricted Early Expression of FABP4. PLoS One *8*, 1–15.

Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene expression data analysis. Genome Biol. *19*.

Wong, B.W., Zecchin, A., García-Caballero, M., and Carmeliet, P. (2018). Emerging Concepts in Organ-Specific Lymphatic Vessels and Metabolic Regulation of Lymphatic Development. Dev. Cell *45*, 289–301.

Wong, V.W., Sorkin, M., Glotzbach, J.P., Longaker, M.T., and Gurtner, G.C. (2011). Surgical approaches to create murine models of human wound healing. J. Biomed. Biotechnol. *2011*, 969618.

Wong, V.W., Rustad, K.C., Akaishi, S., Sorkin, M., Glotzbach, J.P., Januszyk, M., Nelson, E.R., Levi, K., Paterno, J., Vial, I.N., et al. (2012). Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. Nat. Med. *18*, 148–152.

Xiao, Y., Woo, W.-M., Nagao, K., Li, W., Terunuma, A., Mukouyama, Y., Oro, A.E., Vogel, J.C., and Brownell, I. (2013). Perivascular hair follicle stem cells associate with a venule annulus. J. Invest. Dermatol. *133*, 2324–2331.

Xie, S., and Skotheim, J.M. (2020). A G1 Sizer Coordinates Growth and Division in the Mouse Epidermis. Curr. Biol. *30*, 916-924.e2.

Xin, T., Gonzalez, D., Rompolas, P., and Greco, V. (2018). Flexible fate determination ensures robust

differentiation in the hair follicle. Nat. Cell Biol. *20*, 1361–1369.

Yang, C.-C.C., and Cotsarelis, G. (2010). Review of hair follicle dermal cells. J Dermatol Sci. *57*, 2–11.

Yang, H., Adam, R.C., Ge, Y., Hua, Z.L., and Fuchs, E. (2017). Epithelial-Mesenchymal Micro-niches Govern Stem Cell Lineage Choices. Cell *169*, 483–496.

Yano, K., Brown, L.F., and Detmar, M. (2001). Control of hair growth and follicle size by VEGF-mediated angiogenesis. J. Clin. Invest. *107*, 409–417.

Yoshida, H., Kunisada, T., Kusakabe, M., Nishikawa, S., and Nishikawa, S.I. (1996). Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. Development *122*, 1207–1214.

Yoshimoto, M., Porayette, P., Glosson, N.L., Conway, S.J., Carlesso, N., Cardoso, A.A., Kaplan, M.H., and Yoder, M.C. (2012). Autonomous murine T-cell progenitor production in the extra-embryonic yolk sac before HSC emergence. Blood *119*, 5706–5714.

Yoshitomi, Y., Ikeda, T., Saito, H., Yoshitake, Y., Ishigaki, Y., Hatta, T., Kato, N., and Yonekura, H. (2017). JunB regulates angiogenesis and neurovascular parallel alignment in mouse embryonic skin. J. Cell Sci. *130*, 916–926.

Zhang, B., and Hsu, Y. (2017). Emerging roles of transit-amplifying cells in tissue regeneration and cancer. WIREs Dev Biol *e282*, 1–14.

Zhang, B., Tsai, P.C., Gonzalez-Celeiro, M., Chung, O., Boumard, B., Perdigoto, C.N., Ezhkova, E., and Hsu, Y.C. (2016). Hair follicles' transit-amplifying cells govern concurrent dermal adipocyte production through sonic hedgehog. Genes Dev. *30*, 2325–2338.

Zhang, B., Ma, S., Rachmin, I., He, M., Baral, P., Choi, S., Gonçalves, W.A., Shwartz, Y., Fast, E.M., Su, Y., et al. (2020). Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. Nature *577*, 676–681.

Zimmerman, A., Bai, L., and Ginty, D.D. (2014). The gentle touch receptors of mammalian skin. Science *346*, 950–954.

Zomer, H.D., and Trentin, A.G. (2018). Skin wound healing in humans and mice: Challenges in translational research. J. Dermatol. Sci. *90*, 3–12.